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THE ASPERGILLUS GLAUCUS GROUP

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INTRODUCTION

Micro-organisms that successfully develop universality of distribution and omnivorousness of habit generally belong to groups characterized by great diversification in appearance, in morphology, and in physiological adaptation. Because of this diversity investigators, separated in time and distance, encounter organisms varying in minor details and describe them as new. Of the fungi that are universally distributed and significant in incipient spoilage of organic products useful to man, members of the *Aspergillus glaucus* group are among the most important in their action and striking in their habit. Something of the range of conditions under which these molds grow may be illustrated by listing a representative series of products in which they predominate: Improperly dried herbarium specimens (the classic habitat longest reported), "moldy" samples of jellies, jams, soft sugars, honey, dry bread, fruit cake, corn meal, cured meats, cotton and woolen fabrics, leather goods, grain, fodder, or softwoods stored under humid conditions; in fact, almost any organic product if only 1 or 2 percent above the stability percentage in water content may be expected to show yellow perithecia of these species. The smaller the excess of water over the percentage necessary for actual stability, the surer that the mold present will be one of the *A. glaucus* group.

As excess water increases, first other molds, then bacteria, appear and complicate the decomposition process. The presence of any of these organisms is evidence of the earliest stages of decomposition, although fortunately materials rendered moldy by these forms in pure culture do not appear to be poisonous.

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In nature, molds of this group appear as patches of green, yellow, reddish, or reddish-yellow mold and may consist either of (1) a thin network of hyphae bearing scattered yellow perithecia and green conidial heads or (2) a dense mat of mycelium bearing these structures and forming a cushionlike layer.

HISTORICAL BACKGROUND

The molds of this group are world-wide in distribution and omnivorous in habit, hence have been widely studied and described. Botanical reference to these fungi seems to begin with Micheli (*20*)[1] who, in 1729, used the generic name *Aspergillus* (rough head) for the conidial heads characterized by divergent chains of spores and commonly present on badly dried herbarium specimens. Later in the century, Wiggers (*36*) proposed the name *Mucor herbariorum* for the yellow perithecia found mixed with the *Aspergillus* heads, which he regarded as a different mold. In 1809, Link (*17*) designated the green heads *Aspergillus glaucus* and the yellow perithecia *Eurotium herbariorum*. In 1854, De Bary (*7*) proved that the *Aspergillus* heads and *Eurotium* perithecia were borne upon the same mycelium, hence were one fungus.

Recognition of varieties had begun with Corda (*12*), who proposed var. *repens* (Icon. 5, p. 53, 1842). This variety was discussed by De Bary (*7*) in 1854, but he does not seem to have called it a species until 1870 (*8*). At that time he recorded that the ascospores of the *Eurotium* cultures in his possession were markedly different in size. He therefore separated the small-spored series with lenticular, smooth-walled ascospores about 5μ in long axis as *Eurotium repens* De Bary, placing all of the large ones in *Eurotium Aspergillus glaucus* and recognizing *E. herbariorum* Lk. as synonymous. Thus the matter stood for over 30 years.

In 1902, Spieckermann and Bremer (*27*) separated a small-spored strain as *Aspergillus ruber*. Mangin (*18*) in 1909 faced the question, What is *Aspergillus glaucus*? and sought to answer it by a study of herbarium and culture material from many sources. In his conclusions he again followed De Bary in recognizing forms with large and small ascospores. He divided the large forms into two series: "major," with ascospores more than 7.5μ in long axis, and "minor," with ascospores 6.6μ to 7.5μ in long axis. He then described *Eurotium amstelodami* and *E. chevalieri* as separate from *E. repens*, but left most of the questions encountered in the culture laboratory unanswered. Later authors described what they had before them upon specimens or in culture mostly without adequate comparative study of related forms (*3, 4, 5, 9*). Thom and Church in 1926 (*31*) followed Mangin's plan of using the ascospore as the most stable unit of morphology in grouping strains into species aggregates. The accumulation of new material including representative cultures of several new species since that time puts so much confusion into the situation that a restudy of the whole group represented in *Eurotium* is needed.

Aspergillus glaucus or *Eurotium herbariorum*? Although it could be maintained that the name *Eurotium* (designating the perfect stage) should take precedence over *Aspergillus* (descriptive of the conidial apparatus), most recent authors have tended to go back to Micheli and use the name *Aspergillus* for the whole group, because of obvious relationship of many conidial forms for which no perithecia are known.

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[1] Italic numbers in parentheses refer to Literature Cited, p. 44.

SOURCE OF CULTURES

The present study embraces comparative culture and microscopic examination of the strains of this group in the authors' collection. In addition to the isolations made by the authors over a period of 35 years, this collection includes strains and groups of strains received from widely separated collectors together with the strains of this group available from the Centraalbureau voor Schimmelcultures, Baarn, Holland; from the National Collection of Type Cultures, London; and from the London School of Tropical Medicine. Thom and Church in their monograph (*31*) reviewed the taxonomic literature of this group and suggested the placement of all species described before 1925. Those names assigned as synonyms and those that have been found in use on cultures distributed are accounted for here as accurately as possible. Many names at some time applied to material belonging to the group are not now recognizable; if desired for historical purposes, they may be found in the monograph.

GROUP CHARACTERIZATION

All members of this group have certain common characters: Perithecia yellow, globose or subglobose, 75μ to 150μ in diameter, thin-walled, suspended in networks of red or yellow hyphae, borne at or above the surface of the substratum (see figs. 3 and 7), separate or aggregated, few or abundant; producing abundant asci, usually ripening within 2 to 4 weeks, containing 8 ascospores without determined arrangement; ascospores lenticular, generally showing an equatorial line or furrow with or without flanking ridges or crests (see figs. 4 and 12), with wall separating into valves or hemispheres on opposite sides swollen cell in germination.

Conidial heads produced more or less abundantly, radiate to somewhat columnar (see figs. 5, 7, and 11), typically in some shade of green; stalks smooth-walled, septate, terminating in domelike vesicles (see figs. 5 and 11); sterigmata in one series, rather coarse; bearing loosely radiating chains of conidia elliptical to subglobose, uniformly and characteristically roughened.

Aerial hyphae encrusted with yellow to orange or red granules are abundant in perithecial areas of most of the strains of the group. Both cultures and naturally moldy specimens frequently show this as the most conspicuous character, one which is readily recognized with the hand lens.

CULTIVATION AS A BASIS FOR DESCRIPTION

The pattern and size of the ascospore is especially significant in describing species of the *Aspergillus glaucus* group. Nevertheless the conidial apparatus and the vegetative mycelium of particular subgroups become so important that pure culture under known conditions is always needed.

Search for favorable culture conditions under which the members of this group can be described has led the authors to follow Dale (*14*) in the use of high percentages of sucrose in the substratum. Numerous series of comparative cultures were made upon agar media including Czapek's solution with 3 percent, 5 percent, 20 percent, and 40 percent of sucrose, wort, potato-dextrose, Sabouraud's, and malt extract (fig. 1). Of these media, Czapek, containing 20 percent sucrose,[2] has been most useful in making such comparisons, although two species, *Aspergillus medius* and *A. carnoyi*, grow slowly at this concentration but richly at 40 percent sucrose. The comparative growth of three selected species of the group upon 20 percent sucrose Czapek agar and three agar media more commonly employed in mycological work (viz, malt-extract,[3] potato-dextrose,[3] and Czapek's solution agars) is shown in figure 1. The character of the colony as well as the amount of growth is strongly influenced by the culture medium, and it is only upon substrata characterized by high osmotic tension that typical perithecia and conidial heads are produced. In the cultures illustrated in figure 1 this condition obtains only upon 20 percent sucrose Czapek agar. It should be noted, however, that characteristic heads and perithecia normally develop, although few in number, in situations where less concentrated media dry out rapidly, as at the edge of an agar slant.

Colony comparisons for correct identification can best be made in Petri-dish cultures in which direct observation with the compound microscope is feasible.

Incubation at 22° to 25° C. has permitted the development of satisfactory colonies for descriptive study, although the optimum for certain species is above or below this range as will be noted in connection with these descriptions and in the section on the influence of temperature in the general discussion. Colony descriptions have been made upon 3-week-old cultures, except as otherwise stated.

CHARACTERIZATION FROM CULTURE

Mangin (*18*) began his consideration by dividing the group arbitrarily on the basis of ascospore size. He applied the name *Aspergillus herbariorum* to the whole lot having ascospores more than 6.6μ in long axis, further dividing this into a series *major* with ascospores longer than 7.5μ, and a series *minor* with ascospores from 7.5μ to 6.6μ long. He then recognized three species with ascospores still smaller. Thom and Church (*31*) followed Mangin in considering first the large-spored forms. Among a great number of strains examined since 1926, very few large-spored strains have been found. The same observation has been made by George Smith [4] at the London School of Tropical Medicine where this group has also been extensively collected and studied (*1, 16, 23*).

The rarity of cultures showing large ascospores in these collections and the great abundance of small-spored strains have convinced the

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[2] Czapek's solution agar with 20 percent of sucrose: Water 1000 cc., sodium nitrate 3 gm., dibasic potassium phosphate 1 gm., magnesium sulfate 0.5 gm., potassium chloride 0.5 gm., ferrous sulfate 0.01 gm., sucrose 200 gm., and agar 12.5 gm.

[3] Prepared according to formulas employed and published by the Digestive Ferments Company (*15*).

[4] Private communication in the files of the Division of Soil Microbiology, Bureau of Plant Industry.

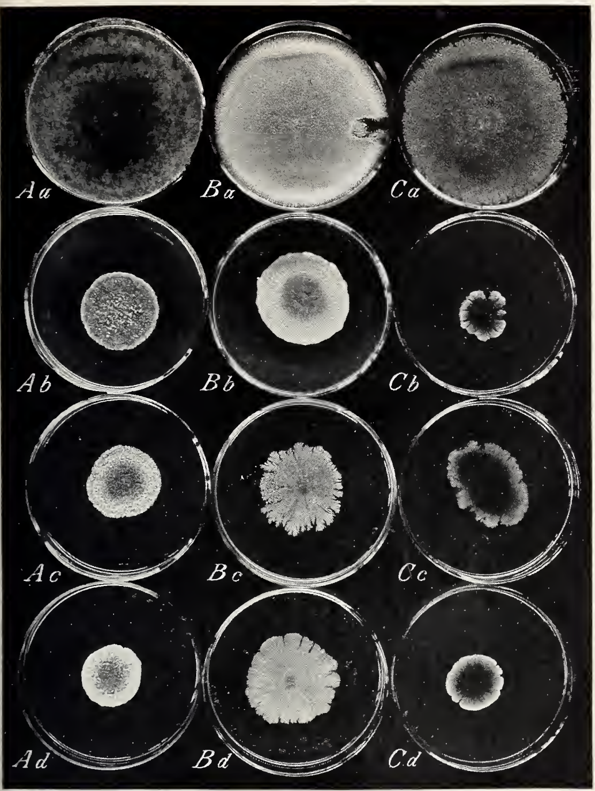

FIGURE 1.—Comparative growth of *Aspergillus repens*, *A. chevalieri*, and *A. ruber* upon four different culture media; 3 weeks' incubation at room temperature. *A*, *a-d*, *A. repens*, No. 5305.5, upon Czapek's solution agar with (*a*) 20 percent of sucrose, (*b*) Czapek's solution agar (3 percent sucrose), (*c*) potato-dextrose agar, and (*d*) malt-extract agar, respectively. *B*, *a-d*, *A. chevalieri*, No. 4125.3; and *C*, *a-d*, *A. ruber*, No. 5599B, upon the same media in similar arrangement.

authors that the latter are invariably the most successful representatives of the group ecologically. Therefore, in the present discussion the small-spored forms will be considered first.

Certain other groups of Aspergilli present characters suggesting those of the *Aspergillus glaucus* group considered here. The ascospores of *A. fischeri* Wehmer and of the *A. nidulans* group (*32*) in general resemble those of the *A. glaucus* group, but the yellow perithecia suspended by yellow and red encrusted hyphae do not occur outside of this group. Conidial heads resembling diminutive members of the *A. glaucus* group are found in the nonascosporic series represented in collections by cultures of *A. gracilis* Bain., *A. conicus* Bloch., and *A. restrictus* George Smith.

The key to species and species aggregates, based primarily on the ascospores, follows.

- Perithecia present.
  - Ascospores lenticular, 6μ or less in long axis.
    - Ascospores with convex faces smooth (or nearly so).
      - Equatorial ridges lacking, furrow absent or showing only as a trace *A. repens* series.
      - Equatorial ridges low and rounded, furrow broad and shallow. *A. ruber* series.
      - Equatorial ridges thin and flexuous, crestlike (spore resembling a pulley) *A. chevalieri* series.
    - Ascospores with convex faces rough *A. amstelodami* series.
  - Ascospores lenticular, 6μ or more in long axis.
    - Conidial heads green.
      - Asci ripening within 4 weeks.
        - Ascospores 6.5μ–7.5μ in long axis *A. minor.*
        - Ascospores 7.5μ–8.5μ in long axis *A. umbrosus.*
        - Ascospores 9.0μ–10.0μ in long axis *A. echinulatus.*
      - Asci ripening slowly, 2 to 3 months, colonies favored by 40 percent sugar.
        - Ascospores with equatorial ridges and furrow *A. medius.*
        - Ascospores usually without equatorial ridges and furrow. *A. carnoyi.*
    - Conidial heads white *A. niveo-glaucus.*
- Perithecia absent *A. argillaceus.*

ASPERGILLUS REPENS SERIES

Ascospores lenticular, mostly 4.8μ to 5.4μ by 3.8μ to 4.4μ, smooth-walled, with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow, but without crests or ridges.

The *Aspergillus repens* series as based upon the ascospore described includes a great number of universally distributed strains which retain some cultural individuality. Consequently several of them have been described as species by earlier workers. From comparison of a great series of these forms, it seems necessary to bring together under the name *A. repens* (Cda.) De Bary, a very considerable number of forms (some of them regarded as species by others) in which the ascospore is typical for the group and the colony difference falls within lines of quantitative rather than qualitative variation.

The following key is offered as a means of separating culturally distinct strains or groups of strains:

- Conidial heads large, borne above the surface layer of perithecia and enveloping hyphae.
  - Heads radiate, long-stalked *A. repens*, No. 4194, 5305.5.
  - Heads columnar, short-stalked *A. dierckxii*,[5] No. 5479.A33.
- Conidial heads small, enmeshed with the perithecia in a felt of sterile hyphae.
  - Felt orange-yellow, loose-textured, radially wrinkled. *A. pseudoglaucus*, No. 5343.
  - Felt yellow-buff, close-textured, plane or nearly so *A. profusus*,[5] No. 5479.A27.

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[5] Species name not recognized as valid by authors of this publication.

Aspergillus repens (Cda.) De Bary. Abhandl I. Senkenberg. Natürf. Gesellsch. 7: 379. 1870.

Synonymy: *A. glaucus* var. *repens* Cda., Icones Fungorum 5: 53, TII, fig. 24. 1842.
A. scheelei Bain. and Sart., Soc. Mycol. de France, Bul. Trimest. 28: 257–262, pl. X. 1912.
A. B var. *scheelei* Bain. and Sart., Soc. Mycol. de France, Bul. Trimest. 28: 262–267, pl. XI. 1912.

Colonies upon Czapek's solution agar (3 percent sucrose) more restricted, plane or somewhat wrinkled, forming a rather compact felt (fig. 1, *A*, *b*), with the marginal area near Scheele's green [6] from developing heads, older areas yellow green to greenish gray and enmeshing large numbers of aborted perithecia producing few ascospores; normal perithecia found only when such colonies spread over the bare walls of the vessel. Reverse in shades of greenish yellow at colony margin to deep maroon or almost black in older areas.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading broadly and rapidly, plane or slightly wrinkled, orange yellow, commonly characterized by broad zones of dull-green conidial heads (fig. 2, *A* and *B*); surface growth consisting of loosely woven hyphae studded with orange granules enmeshing abundant yellow perithecia above which project abundant conidial heads, the whole colony and especially the marginal areas and adjacent wall of the culture dish commonly overgrown by a loose aerial network of hyphae bearing conidial heads and scattered perithecia; reverse varying from yellow orange to deep maroon.

Perithecia very abundant, borne in loose network of yellow to orange-red hyphae (fig. 3, *A*), yellow, spherical to subspherical, mostly 75μ to 100μ, occasionally up to 125μ; asci 10μ to 12μ; ascospores lenticular, mostly 4.8μ to 5.6μ by 3.8μ to 4.4μ, smooth-walled, with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges (fig. 4, *A*). Conidial heads abundant, varying in different strains from 125μ to 175μ in diameter, consisting of diverging chains of conidia radiating from a hemispherical vesicular apex of the stalk (fig. 5, *A*); stalks smooth, mostly colorless, 500μ to 1,000μ in length, broadening at the apex to a vesicular area, about 25μ to 40μ in diameter; sterigmata in one series 7μ to 10μ by 3.5μ to 4.5μ; conidia elliptical to subglobose, spinulose, mostly 5μ to 6.5μ.

Represented by cultures No. 4194, No. 5305.5, and more than a score of others included in this study. In this connection it should be noted that of 37 cultures examined in the present study that produced ascospores characteristic of the *A. repens* series, 29 produced colonies and microscopic details that place them in the species *A. repens* as described.

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[6] For colony descriptions Ridgway's Color Standards and Nomenclature (24) has been employed as a reference standard.

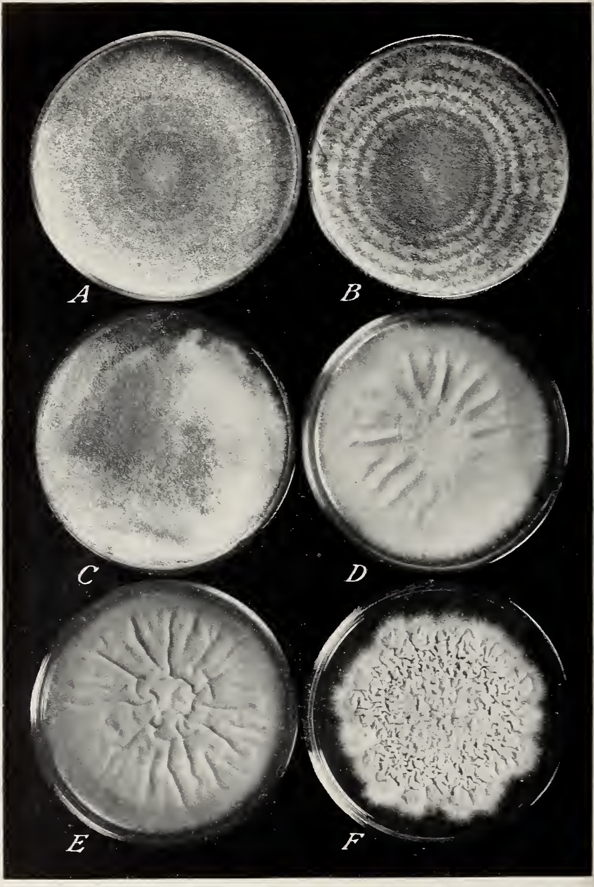

FIGURE 2.—Comparative growth of members of the *Aspergillus repens* series upon Czapek's solution agar containing 20 percent of sucrose; incubation at room temperature for 3 weeks: *A* and *B*, Typical cultures of *A. repens; C*, No. 5479.A33 received as *A. dierckxii; D*, No. 5479.A27 received as *A. profusus; E, A. pseudoglaucus* No. 5343; *F*, No. 5208.e22. See descriptive notes pp. 6, 7.

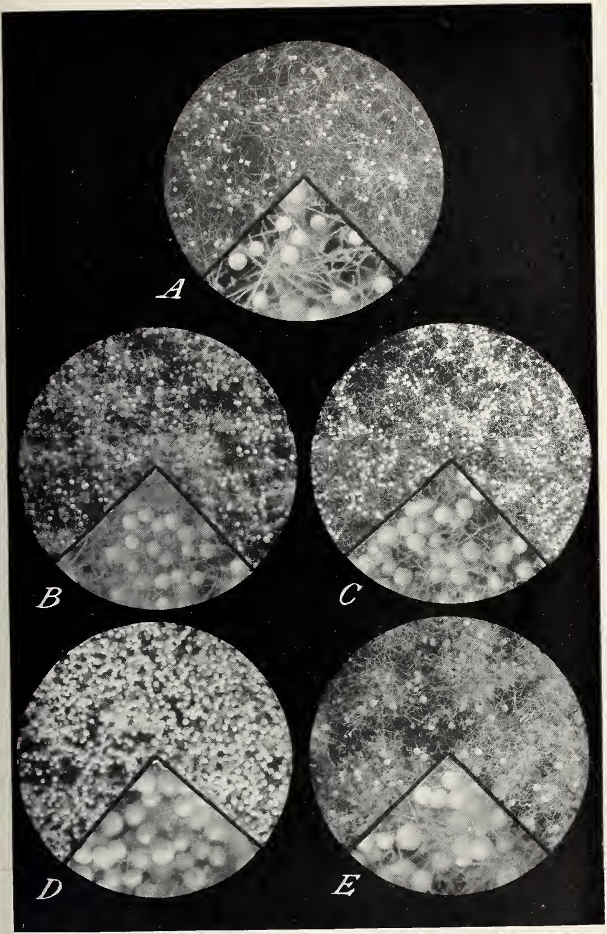

FIGURE 3.—Marginal areas of colonies of representative species of the *Aspergillus glaucus* group showing the relative size and arrangement of perithecia (conidial heads not in focus): *A*, *Aspergillus repens*; *B*, *A. chevalieri*; *C*, *A. ruber*; *D*, *A. amstelodami*; *E*, *A. echinulatus*. Figures ×10; inserts ×30.

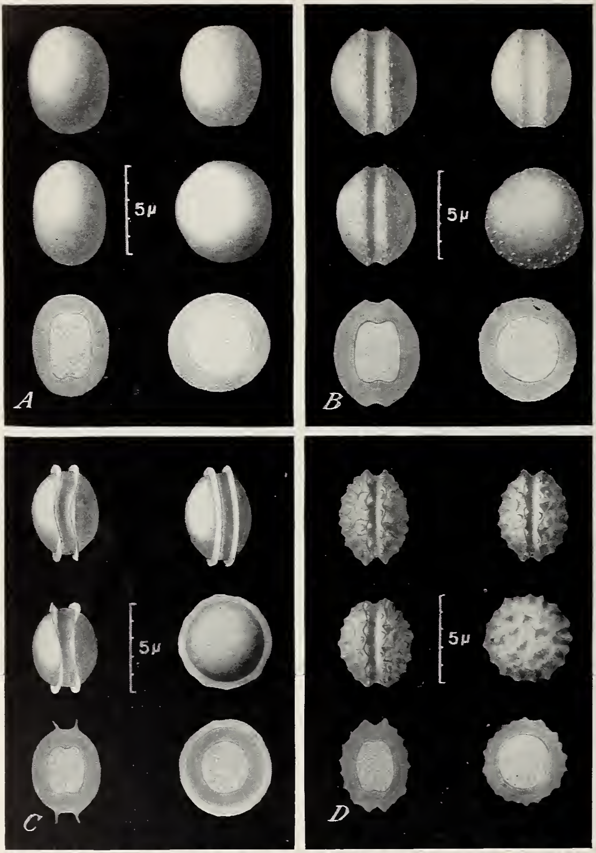

FIGURE 4.—Ascospores representative of the four small-spored series of the *Aspergillus glaucus* group: *A*, *A. repens; B*, *A. ruber; C*, *A. chevalieri;* and *D*, *A. amstelodami*. In each species upper left and right and center left spores represent surface, profile views; center right, surface in face view; lower left, optical section in profile; and lower right, optical section in face view.

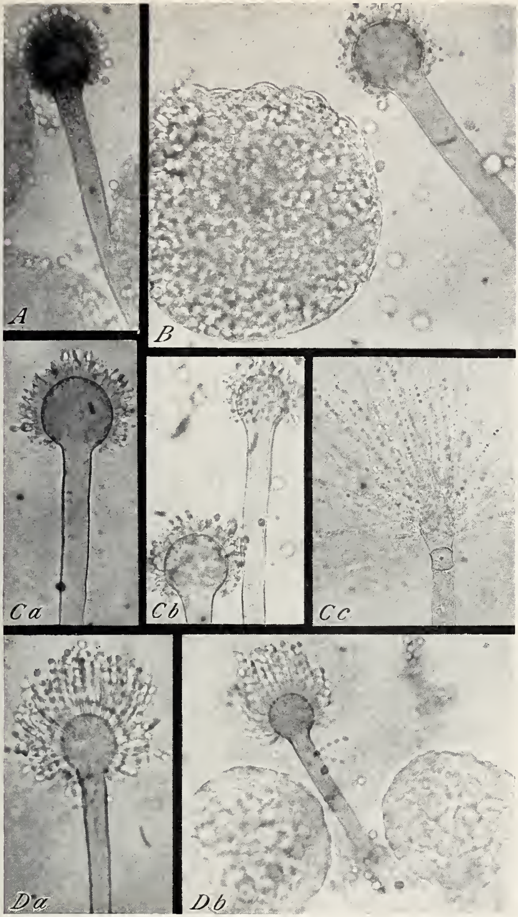

Figure 5.—*A*, *Aspergillus repens*, typical conidial head and fractions of three perithecia. *B*, *A. chevalieri*, conidial head and mature perithecium filled with ascospores. *C*, *A. ruber:* ***a*** and *b*, Typical heads, the former showing clearly the single series of sterigmata characteristic of the "glaucus" *Aspergilli;* ***c***, atypical head in which each sterigma has produced a secondary head or vegetative hypha. *D*, *A.* ***amstelodami:*** *a*, Typical conidial head; *b*, smaller conidial head and two young perithecia. ×400.

This description is manifestly broad enough to include strains approximating the description given by Bainier and Sartory for *Aspergillus scheelei* and *Aspergillus B* var. *scheelei* (*5*). Evidently *A. scheelei* was thought by the describers to represent a species with somewhat larger ascospores showing a more definite furrow, whereas *Aspergillus B* var. *scheelei* was a strain with smaller ascospores almost without a trace of furrow. Both species were described as characterized by the production of a yellow pigment. In the authors' experience, a distinction based upon color is largely invalidated by variants bridging the whole range from yellow orange to deep orange red and even shades of brown when large numbers of strains of this series are compared in culture. Strains also vary slightly in the pattern of their ascospores, some rarely producing spores with a trace of furrow and others bearing a large proportion with such traces. But among spores of a single strain limited variation in this character is normally encountered. Thus the presence or absence of a slight furrow, unless accompanied by significant differences in morphology or colony character, would not seem to justify specific descriptions in this series.

A strain designated as *Aspergillus dierckxii*, presumably by Biourge but thus far unpublished, was included in Gould and Raistrick's study of pigment production in the *A. glaucus* group (*16*). As received from Raistrick's laboratory, this organism (the authors' No. 5479.A33) produces colonies showing no zonate arrangement of conidial heads (fig. 2, *C*). Further, heads are borne on shorter stalks than in typical *A. repens* and consist of columns of conidia rather than radiating chains. Little or no red color appears in the colonies or in reverse. Although no other strains showing exactly these differences have appeared in the authors' collection, separation as a distinct species is believed unwarranted.

Aspergillus pseudoglaucus Bloch. emend. Ann. Mycol. 27: 207. 1929.

Colonies upon Czapek's solution agar (3 percent sucrose) restricted in growth, radiately wrinkled, yellow green to shades of gray, consisting of a mixture of small conidial heads, young or aborted perithecia and more or less colorless hyphae; reverse orange at center becoming lighter toward the margin.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading, strongly wrinkled in a predominantly radiate manner, consisting of a felt of orange-encrusted hyphae enmeshing abundant perithecia, orange except at margin where yellow green predominates from the presence of small conidial heads admixed with perithecia in the mycelia felt (fig. 2, *E*); reverse yellow becoming orange brown or maroon in marginal areas.

Perithecia abundant, spherical to subspherical, mostly 60μ to 80μ though occasionally 100μ in diameter, yellow, embedded in a felt of orange mycelium; asci 10μ to 12μ in diameter; ascospores lenticular, 4.6μ to 5.2μ by 3.6μ to 4.0μ, occasionally 5.6μ in long axis, smooth-walled, with equatorial region rounded or flattened, without ridges, and with furrow generally lacking though occasionally showing as a trace. Conidial heads few in number and generally submerged in the mycelial felt, small, mostly 50μ to 75μ in diameter but occasionally up to 100μ; stalks mostly 150μ to 300μ in length, 5μ to 8μ at the base, broadening to a terminal vesicle 12μ to 20μ in diameter; sterigmata in a single series, 6μ to 8μ by 3μ to 4μ; conidia subglobose, delicately spinulose, variable in size ranging from 5.5μ to 7.5μ in diameter.

Represented in the authors' collection by No. 5343 received from Baarn as *A. pseudoglaucus* Blochwitz and No. 5479.A38 received from George Smith as *A. fumigatoides* Bain. and Sart.

There is reason to believe that the former culture is directly derived from Blochwitz's type. It becomes necessary therefore to emend the description given by him insofar as the measurements and markings of ascospores and conidia are concerned. Gould and Raistrick (*16*) reported biochemical data upon a culture, No. A 38, received from Biourge as *A. fumigatoides* Bain. and Sart. (No. 5479.A38 of the present collection), which is identical with *A. pseudoglaucus* (No. 5343) as sent to the authors by Westerdijk. Obviously the culture from Biourge is incorrectly named. It does not fit the species description nor the figures of *A. fumigatoides* (*2*) in the size of its conidia, the character of its perithecial wall, or the pattern of its ascospores. The ascospores of *A. fumigatoides* are shown as roughened over their entire surfaces, as in *A. fischeri*, whereas those of No. 5479.A38 certainly belong in the *A. repens* series. Distinctive colony characters, however, maintained stably through many transfers, together with the size of the conidial apparatus, warrant separating *A. pseudoglaucus* from *A. repens* and maintaining it as a species.

A strain, No. 5208.e22, received from Dr. B. O. Dodge and Miss Marjorie E. Swift, of the New York Botanical Garden, in 1931, is characterized by an intensely wrinkled colony and a further reduction in the size and number of conidial heads. Colonies are dull orange red and bear abundant perithecia enmeshed in a close felt of sterile encrusted hyphae. Obviously it should be considered with *A. pseudoglaucus*.

In cultures received from Baarn (No. 5633.16) and from George Smith (No. 5479.A27) as *A. profusus* Hann (nomen nudum) there is a pronounced accentuation of the floccose habit already noted in *A. pseudoglaucus*. Upon 20 percent sucrose Czapek agar these cultures, which are obviously duplicates, produce spreading, plane or radiately wrinkled, floccose colonies consisting of a close felt of light tan to buff-colored hyphae, bearing occasional perithecia and widely scattered conidial heads. The perithecia are commonly embedded deep within the felt, whereas the conidial heads are most evident at the colony margin. Although the ascospores of these strains are definitely of the *A. repens* type, they are generally flattened along their equators and commonly show a trace of furrow. An occasional ascospore shows a minute roughness in the equatorial region. The differences observed do not seem to warrant perpetuating the name *A. profusus*, and in agreement with Dr. Westerdijk and coworkers (*11*), the cultures have been assigned to *A. pseudoglaucus*.

Culture No. 4724.46, received from Raistrick in 1923 as *Aspergillus novus* Wehmer (nomen nudum) bears ascospores duplicating those of the cultures just considered. This strain is of particular interest, because in routine transfers colonies of two distinct types commonly appear. One of these is predominantly floccose and suggests the colonies of the strains received as *A. profusus*. The other consists of a crowded surface layer of perithecia, which is thinly veiled by a loose felt of orange-red hyphae, and in its gross appearance, with the exception of its lighter color, is strongly suggestive of certain cultures of *Aspergillus ruber*. The authors agree with Wehmer (*35*) and Blochwitz (*9*) that the species designation, *Aspergillus novus*, should be withdrawn.

A nonascosporic culture distributed by Biourge as *Aspergillus argillaceus* n. sp., was received upon two occasions from Prof. Raistrick's laboratory. From its appearance in culture and the morphology of its conidial structures this fungus would seem to represent a member of the *Aspergillus repens* series in which perithecial development has been wholly suppressed. Although it is questionable whether this fungus represents a true species, a brief description is given because of its inclusion in biochemical studies by Raistrick and coworkers (*1*, *16*, *23*). Colonies upon Czapek's solution agar with 20 percent of sucrose spreading irregularly, consisting of a loose floccose felt of aerial hyphae and abundant conidial heads, pale yellow green to clay color; reverse yellow. Upon Czapek's solution agar (3-percent sucrose) colonies restricted, raised in center, thinning toward margin, consisting of abundant conidial heads and interlacing hyphae, buff to clay colored; reverse yellow to tawny. Conidial heads abundant, dull green, up to 200μ diameter and commonly splitting into fairly well defined columns, stalks up to 1,000μ in length. Conidia subglobose mostly 5.5μ to 6.0μ but occasionally up to 7.0μ in long axis, spinulose.

ASPERGILLUS RUBER SERIES

Ascospores lenticular, 5.0μ to 6.0μ by 4.0μ to 4.8μ, colorless, with broad, shallow furrow generally evident and flanked by low ridges, and with walls smooth except for minute roughness along the equatorial ridges.

This series includes a great number of strains showing variations in cultural appearance but producing ascospores of a limited size and fairly well defined pattern. For this particular study some 30 strains, received from various culture collections and contributors and selected from the isolations made in this laboratory over a period of many years, have been chosen for repeated culture and examination. Among these, many strains appear distinct, but their differences are commonly bridged by intermediate forms. Separation within the series, therefore, must be along one of the following lines—either (1) strains must be separated upon minor characters, such as differences in the intensity of pigmentation, slight variations in ascospore character, etc.; or (2) strains must be set off in broad and elastic subgroups, in some cases including large numbers which vary appreciably in detail. The second alternative is desirable, for the first can lead only to increased hairsplitting and end in greater confusion than that which already exists.

Spieckermann and Bremer's designation *Aspergillus ruber* (*27*) is assigned to the series, because its members are predominantly producers of an intense red pigment and bear ascospores of the general size and pattern described by these authors. The only other described fungus possessing a similar ascospore and characterized by its red color is Bainier and Sartory's *Aspergillus sejunctus* (*3*).

Although it is now quite impossible to say what particular fungus either pair of investigators had at hand, both are believed to have worked with members of the large series now under consideration. *Aspergillus ruber* is retained, as it is more descriptive of the series in addition to being the prior species.

To accentuate cultural similarities and differences between the strains studied, a wide variety of culture media has been employed. Based upon their appearance in culture, the strains fall into a few well-defined subgroups (see figs. 6 and 7). The strains belonging to one of these fairly represent the fungus described by Spieckermann and Bremer (*27*) and are at the same time most abundant in the entire series, hence are considered as typical of *A. ruber* (see figs. 6, *A*, and 7, *A*). The general characters of the remaining subgroups are listed to show the extreme cultural variation that is to be expected within the series, but specific names are withheld, because to perpetuate or propose such would multiply rather than clarify the confused nomenclature of this abundant and variable series of organisms.

To illustrate more definitely the variation that occurs, the following outline of possible lines of separation is inserted:

- Colonies predominantly perithecial.
  - Colonies red, perithecia abundant in a layer at the agar surface and overgrown by a felt of red encrusted hyphae ... *A. ruber*, No. 5599B, 5526.H7.
  - Perithecia abundant in a loose, floccose overgrowth of red hyphae as well as in a layer at the agar surface ... No. 134.
  - Colonies thin, orange red, perithecia abundant in old cultures and on very concentrated media ... No. 5114.
- Colonies predominantly conidial.
  - Colonies gray, heads long stalked, perithecia few ... No. 5629E.
- Colonies mixed conidial and perithecial.
  - Colonies orange red and green, zonate, perithecia borne at the agar surface and piled in a loose network of superficial hyphae ... No. 5600A.
- Colonies predominantly floccose, colonies red, brown, perithecia and conidial heads few ... *A. lovainensis*,[7] No. 5479.A31.

Aspergillus ruber (Spieck. and Brem.) Thom and Church (*31, p. 112*).

Synonymy: *Eurotium rubrum* Spieck. and Brem., Landw. Jahrb. 31: 92. 1902.
A. sejunctus Bain. and Sart., Soc. Mycol. de France, Bul. Trimest. 27: 361–367, pl. XI. 1911.

Colonies upon Czapek's solution agar (3 percent sucrose) more restricted, plane (fig. 1, *C*, *b*), orange brown to red brown in color; perithecia generally abundant though often abortive; conidial heads pea green to olive green, abundant in some strains, few and largely vestigial in others; reverse orange red to maroon.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading rapidly and broadly in a regular manner or unevenly, plane, predominantly red, ranging from ferrugineous to morocco red; perithecia very abundant, borne in a dense layer at the agar surface and largely concealed within and beneath a close-textured felt of red-encrusted hyphae; conidial heads projecting above the felt, pale gray green to deep olive gray, more or less abundant, and generally crowded near the center or scattered unevenly over the colony (figs. 6, *A*, and 7, *A*); reverse in shades of dark red brown.

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[7] Species name not recognized as valid by authors of this publication.

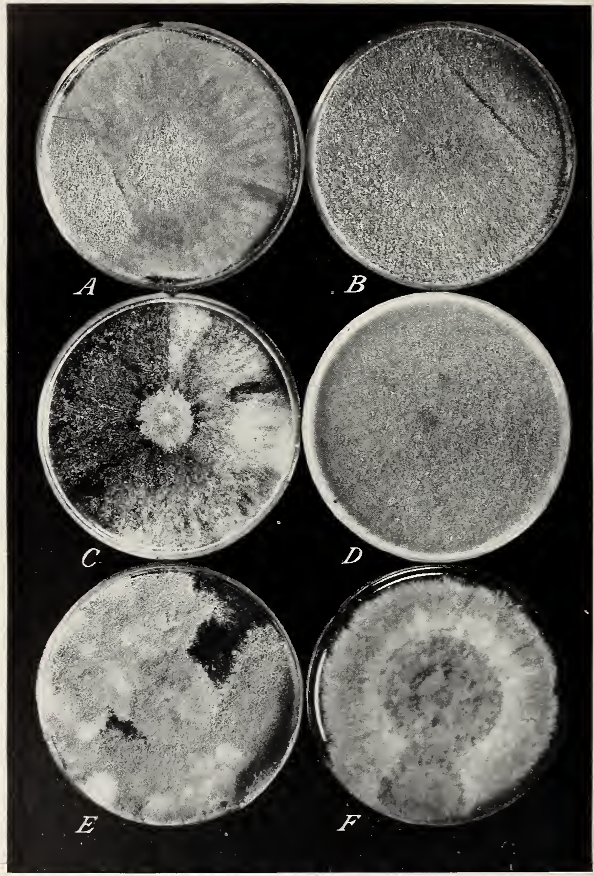

FIGURE 6.—Different colony types developed in the *Aspergillus ruber* series in 3 weeks at room temperature upon 20 percent sucrose Czapek agar: *A*, Typical *A. ruber*, represented by No. 5599B and similar cultures; *B*, No. 5600A; *C*, No. 5114; *D*, No. 134; *E*, No. 5629E; and *F*, No. 5479.A31. Compare figure 7 and see descriptive notes pp. 14, 15.

Perithecia very abundant, largely enmeshed in a felt at the agar surface (fig. 7, *A*), yellow to orange red, spherical to subspherical, mostly 80μ to 120μ though occasionally up to 140μ in diameter; asci 12μ to 15μ; ascospores lenticular, 5.2μ to 6.0μ by 4.4μ to 4.8μ, with furrow generally evident as a broad and shallow depression around the spore equator, ridges low and often inconspicuous, walls smooth except for minute roughness along equatorial ridges (fig. 4, *B*). Conidial heads generally abundant, numerous in localized areas or scattered thinly over the colony, pale blue green, radiate, 150μ to 250μ in diameter; stalk smooth, colorless to orange brown, 500μ to 750μ in length,

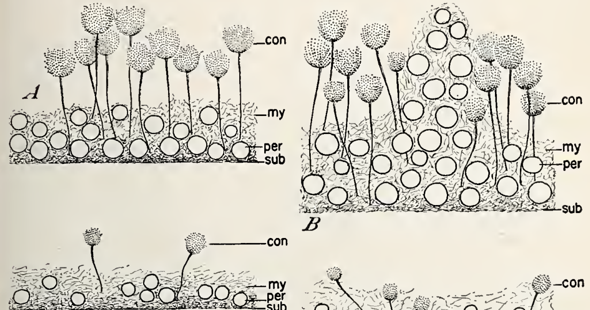

FIGURE 7.—Diagrammatic representation of cross sections of different colony types in the *Aspergillus ruber* series developed at room temperature upon 20-percent sucrose Czapek agar, showing the relative abundance and disposition of conidial heads (*con*) and perithecia (*per*), and the amount and character of the mycelium (*my*) above the substratum (*sub*): *A*, Typical colony of *A. ruber* as seen in No. 5599B; *B–F*, atypical colonies as seen respectively in No. 5600A, No. 5114, No. 134, No. 5629E, and No. 5479.A31. See descriptive notes pp. 15, 17–18; see also figure 6. Scale approximate.

broadening to 14μ to 16μ where it passes into the subglobose vesicular area of 25μ to 35μ diameter (fig. 5, *C*, *a* and *b*); sterigmata in a single series 7μ to 9μ by 4μ to 5μ; conidia elliptical to subglobose, closely spinulose, mostly 5μ to 6.5μ in long axis.

Aspergillus ruber is represented in this study by No. 5599B, No. 5612.6, and others. Of 31 strains examined belonging to the whole series, 19 showed colonies and microscopic characters that place them within the species *Aspergillus ruber* as described above. Although the majority of strains belonging to the *A. ruber* series produce plane colonies as noted in the description, occasionally strains may produce colonies more or less wrinkled.

Culture No. 134 (figs. 6, *D*, and 7, *D*) represents a subseries of several strains that differ from the above not only in colony character upon 20 percent sucrose Czapek agar, as indicated in the preceding key, but also in their growth upon media of lower concentration. These grow slowly and poorly on Czapek (3 percent sucrose), potato-dextrose, and wort agars, producing small raised colonies of 1 to 2 cm. in diameter bearing neither normal conidial heads nor perithecia.

Strain No. 5114 (figs. 6, *C*, and 7, *C*) produces abundant perithecia only on very dry areas of the substratum in old cultures or on media containing a sucrose concentration of 40 percent or more. In contrast to other strains, this fungus grows better upon media containing 4 percent agar than the usual 1.2 percent agar, further establishing its xerophytic character.

Strains such as No. 5629E (figs. 6, *E*, and 7, *E*), are occasionally encountered that are predominantly conidial and characterized by rampant hyphae bearing abundant conidial heads piled in floccose masses above the substratum and upon the edges of the culture dish or tube. They thus produce colonies markedly in contrast with the usual *Aspergillus ruber* concept. But the character of their ascospores, together with the occurrence of occasional sectors in colonies of these strains showing the usual mixture of perithecia and conidial heads, relates them definitely with *A. ruber*.

Strain No. 5600A (figs. 6, *B*, and 7, *B*) represents a subsection of the series in which conidial heads are abundant and generally arranged in fairly definite zones and patches with loose clusters of perithecia irregularly and conspicuously distributed among and above the grouped green heads. These strains are further characterized by somewhat larger perithecia than those of No. 5599B, being mostly in the range of 125μ to 150μ in diameter, and by producing less red color in the colonies and their reverse.

Culture No. 5479.A31 (figs. 6, *F*, and 7, *F*) is characterized by a close felt of red-brown hyphae, which completely covers the agar surface and in which scattered perithecia are borne. Conidial heads are scarce and largely confined to the colony margin. This strain, which was included in Gould and Raistrick's study of pigmentation in the *Aspergillus glaucus* group (*16*), was received from George Smith under the name *Aspergillus lovainensis* and attributed to Biourge. Except for its darker color this fungus in culture bears a striking resemblance to one received from Baarn as *Aspergillus profusus* (No. 5633.16), which showed similar floccose habits. However, the ascospores of the latter are smaller and less furrowed and are essentially smooth along the equatorial margin. The degree of relationship between the two is questionable.

ASPERGILLUS CHEVALIERI SERIES

Ascospores lenticular, mostly 4.6μ to 5.0μ by 3.4μ to 3.8μ, occasionally up to 5.2μ in long axis, with walls smooth or slightly rough, with crests prominent, flexuous, often recurved, and with furrow conspicuous but consisting more of a trough between extended equatorial crests than a depression in the spore wall.

Strains belonging to this series show appreciable difference in colony character and to a limited degree in the surface markings of their ascospores. The ascospores of all, however, are characterized by their continuous, prominent equatorial crests which do not form an integral part of the spore wall, but extend well beyond the margin of the spore body proper. To use Mangin's (*18*) exceedingly descriptive term, they are characteristically "pulley-form."

The following key will serve to differentiate groups of strains within the series:

Ascospore walls smooth.
  Crests prominent, thin, flexuous, often recurved .... *A. chevalieri*, No. 4125.3, 5061.
  Crests evident, low, usually erect .... *A. chevalieri* var. *multiascosporus*,[8] No. 5633.26.
Ascospore walls more or less roughened.
  Crests thin, flexuous, often recurved; conidia roughened ..... *A. oriolus*,[8] No.5479.A30.
  Crests thicker, usually erect; conidia smooth .... *A. chevalieri* var. *intermedius* n. var., No. 5612.107.

Aspergillus chevalieri (Mangin) Thom and Church (*31, p. 111*).

Synonymy: *Eurotium chevalieri* Mangin, Ann. des Sci. Nat., Bot. (Ser. 9) 10: 361–362, fig. 12. 1909.

Colonies upon Czapek's solution agar (3 percent sucrose) more restricted, plane, closely felted, bluish gray in center, with typical heads and perithecia largely confined to marginal area (fig. 1, *B*, *b*); reverse maroon in center to orange at margin.

Colonies upon Czapek's solution agar with 20 percent of sucrose growing best at 30° C. or above, spreading, plane to somewhat wrinkled in central area (fig. 8, *A*) with abundant conidial heads in blue-green shades, distributed evenly over the whole surface or more crowded in localized areas, projecting above a layer of abundant perithecia enmeshed in orange-red hyphae at the agar surface; reverse in shades of orange red to brown, more intense in center.

Perithecia abundant and closely enmeshed in a felt of orange-red encrusted hyphae (fig. 3, *B*), mostly 100μ to 140μ, occasionally up to 150μ, globose to subglobose, yellow to orange; asci 9μ to 10μ; ascospores lenticular, 4.6μ to 5.0μ by 3.4μ to 3.8μ, with walls smooth, with equatorial crests prominent, thin and often recurved, and with furrow consisting more of a trough between parallel crests than an equatorial depression in the spore body (fig. 4, *C*). Conidial heads abundant, pale blue green, appearing radiate from divergent conidial chains, mostly 125μ to 175μ in diameter, occasionally larger; stalks mostly 700μ to 850μ in length, enlarging to a vesicular apex, somewhat globose (fig. 5, *B*), 25μ to 35μ in diameter; sterigmata in a single series, closely packed, 5μ to 7μ by 3μ to 3.5μ; conidia subglobose, spinulose, mostly 4.5μ to 5.5μ in diameter.

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[8] Species name not recognized as valid by authors of this publication.

Aspergillus chevalieri is represented in the present study by cultures No. 4125.3, No. 5061, and others. The species name is limited to strains bearing ascospores with smooth walls and prominent, thin equatorial crests because it is believed that these strains most nearly represent the organism described by Mangin (*18*). When grown upon 20 percent sucrose Czapek agar, these strains are further characterized by their predominantly orange-red colonies, pale blue-green conidial heads, and dark-colored reverse. Within the series, different strains vary in the quantity of conidial heads produced, e. g., No. 5061 regularly produces an abundance of heads, No. 4125.3 relatively few.

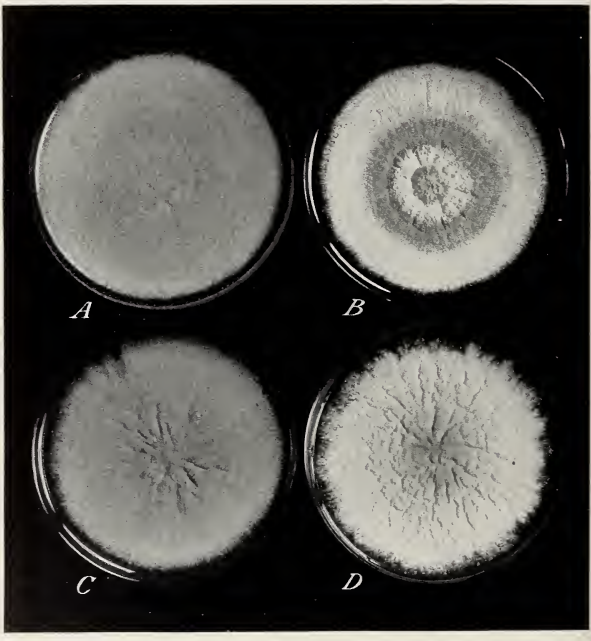

FIGURE 8.—Comparative growth of members of the *Aspergillus chevalieri* series in 3 weeks at room temperature upon 20 percent sucrose Czapek agar: *A*, Typical *A. chevalieri*, No. 4125.3; *B*, No. 5633.26, received as *A. chevalieri* var. *multiascosporus; C*, No. 5479.A30, received as *A. oriolus;* and *D*, *A. chevalieri* var. *intermedius*, No. 5612.107. See descriptive notes pp. 19–22.

Strains of this series are not so commonly encountered as are those of the *A. repens*, *A. amstelodami*, or *A. ruber* series. And within the series, strains that conform with the typical species description are relatively less numerous than in these other series. In the present study 14 strains belonging to the *A. chevalieri* series have been examined and only 6, or less than half, are wholly representative of the species *A. chevalieri.*

The series as a whole seems to be relatively unstable, and certain strains and groups of strains appear transitional between this series and the *A. repens* series on the one hand and the *A. amstelodami* series on the other.

Culture No. 5633.26, received from Baarn as the type of *Aspergillus chevalieri* var. *multiascosporus* Nakazawa, Takeda, Okada, and Simo points toward the *A. repens* series. Its ascospores have smooth walls, as in the typical strains of *A. chevalieri,* but bear low, erect crests in contrast to the thin, flexuous crests characteristic of this species. Spores lacking crests are occasionally seen and these closely resemble *A. repens.* The colony upon 20 percent sucrose Czapek agar is definitely of the character of *A. chevalieri.* Nakazawa and coworkers *(21)* separated it from *A. chevalieri* because of its more floccose habit and its more abundant production of perithecia. The former character is evident in the authors' cultures, but perithecia are not produced more abundantly than in certain strains entirely typical of *A. chevalieri.*

Another variation from the typical species is seen in culture No. 5479.A30 received from George Smith as *Aspergillus oriolus* and attributed to Biourge. The ascospores of this culture (and another that is in the present collection, No. 5633.25) have crests typical of *A. chevalieri,* but the spore walls are finely roughened over their entire surfaces. This character is suggestive of *A. amstelodami,* although the roughening of the wall is slight in comparison with that species. The colony upon 20 percent sucrose Czapek agar is essentially like that of typical strains of *A. chevalieri* but is less red in color and bears fewer conidial heads. Although these cultures can be distinguished from type they are not recognized as warranting separation.

The roughening of the ascospore wall is further accentuated in a group of four apparently similar strains, which it is believed are truly intermediate between *A. chevalieri* and *A. amstelodami.* Because their ascospores bear crests of the *A. chevalieri* type and hence appear "pulley-form" they are retained in the species. However, because they differ from typical strains in additional particulars, they are considered a new variety, namely, *Aspergillus chevalieri* var. *intermedius.*

Aspergillus chevalieri (Mangin) var. **intermedius,** n. var.

Colonies upon Czapek's solution agar with 20 percent sucrose differing from the species in texture and color, and presenting withal a picture intermediate between *A. chevalieri* and *A. amstelodami* (figs. 8, *D*, and 9, *B*). Ascospores lenticular, mostly 4.6μ to 5.2μ by 3.6μ to 4.0μ, occasionally 5.4μ in long axis, with walls roughened and with prominent equatorial crests. Conidial heads dull green, radiate to columnar, mostly 100μ to 125μ in diameter, and up to 175μ in length; conidia elliptical to subglobose, smooth-walled, mostly 3μ to 4μ in long axis.

Represented in this study by culture No. 5612.107, which was received from George Smith as No. 107 and bore the following notation: "Isolated G. S. from cotton yarn, 1927. Close to *A. chevalieri*—differs in having smooth, small conidia and ascospores somewhat larger than type." Duplicated by three additional strains received from European sources.

Aspergillus chevalieri var. *intermedius* appears to be transitional between the *A. chevalieri* and the *A. amstelodami* series. Such a view is supported (1) by the pattern of the ascospores, which shows both the extended and often recurved equatorial crests characteristic of *A. chevalieri* and the rough spore walls of *A. amstelodami;* and (2) by the coloration of the colony as shown in figure 9. *Aspergillus chevalieri* var. *intermedius* upon 20 percent sucrose Czapek agar becomes orange yellow above and orange to light brown in reverse, *A. amstelodami* remains bright yellow with reverse uncolored, whereas *A. chevalieri* becomes red in the colony and reverse. The smoothness of conidia in *A. chevalieri* var. *intermedius* is a distinctive character and appears in neither *A. chevalieri* nor *A. amstelodami*. Although this variety from many points of view appears to be a hybrid, proof of such origin is lacking.

ASPERGILLUS AMSTELODAMI SERIES

Ascospores 4.7μ to 5μ by 3.6μ to 3.8μ, lenticular, colorless, with equatorial furrow conspicuous, broadly V-shaped and flanked by broad irregular ridges, with walls irregularly and unevenly ridged or roughened over the entire surface.

Included in this series are strains that differ greatly in colony appearance. However, their close relationship is demonstrated by the similarity in size and pattern of their ascospores and is further shown by the dark olive-green color of their conidial heads, the bright-yellow color of their perithecia, and the absence of any red either in the colonies or their reverse.

The following key is designed to show the variation that occurs within the series and to offer a means of separating strains or groups of strains that are culturally distinct:

Colonies predominantly perithecial.
  Conidial heads abundant in central area and often in concentric zones. *A. amstelodami*, No. 126, and others.
  Conidial heads widely scattered or lacking .... No. 5644.c.
Colonies predominantly conidial.
  Perithecia widely scattered, superficial .... No. 5667.405.
  Perithecia abundant in a felted layer above the conidial heads. *A. montevidensis*, No. 5290.
Colonies very thin, perithecia and conidial heads widely scattered .... No. 5681.1.

Aspergillus amstelodami (Mangin) Thom and Church (*31, p. 113*).

Synonymy: *Eurotium amstelodami* Mangin, Ann. des Sci. Nat., Bot. (ser. 9) 10: 360–361. 1909.
E. repens var. *amstelodami* Vuill., Soc. Mycol. de France, Bul. Trimest. 36: 131. 1920.

Colonies upon Czapek's solution agar (3 percent sucrose) restricted, 4 to 6 cm. in diameter, plane or closely wrinkled, yellow to dull yellow gray in color from abundant perithecia admixed with sterile hyphae and developing conidial heads; reverse uncolored, becoming tawny in age.

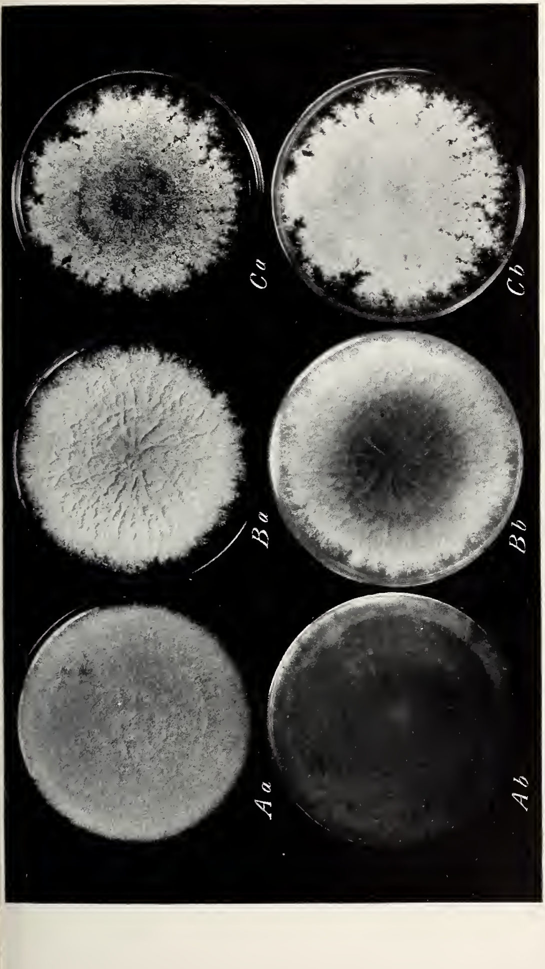

FIGURE 9.—Transitional character of colony of *Aspergillus chevalieri* var. *intermedius*: *A*, *a* and *b*, Colony and reverse respectively of typical *A. chevalieri*, No. 4125.3; *B*, *a* and *b*, the same of *A. chevalieri* var. *intermedius*, No. 5612.107; and *C*, *a* and *b*, the same of typical *A. amstelodami*, No. 126. Three-week-old cultures upon 20 percent sucrose Czapek agar at room temperature.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading, 8 to 10 cm. diameter, more or less wrinkled and zonate (fig. 10, *A* and *B*), perithecia very abundant and clustered in masses forming a dense layer at the agar surface (fig. 3, *D*), bright yellow in color, lending a characteristic appearance to the colony; conidial heads deep olive green, abundant in colony center and scattered more or less unevenly over the whole surface, occasionally obscuring the layer of perithecia beneath. Reverse persistently yellow under perithecial areas, more or less green where conidial areas predominate.

Perithecia globose to subglobose, mostly 115µ to 140µ in diameter, occasionally up to 160µ, not covered by or embedded within a felt of sterile hyphae (fig. 3, *D*); asci mostly 10µ to 12µ, 8-spored; ascospores lenticular, 4.7µ to 5.0µ by 3.6µ to 3.8µ, with prominent V-shaped equatorial furrow and broad irregular ridges, and with walls roughened over their entire surfaces (fig. 4, *D*). Conidial heads radiate-columnar (fig. 5, *D*, *a* and *b*), mostly 120µ to 150µ in diameter, occasionally larger; stalks colorless to pale yellow green, 275µ to 350µ in length, broadening to 10µ to 12µ in diameter below the vesicle; vesicle subglobose, 18µ to 25µ in diameter; sterigmata about 5µ to 6.5µ by 2.5µ to 3.5µ; conidia finely spinulose, subglobose, variable in size, ranging from 3.5µ to 5.2µ, mostly about 4µ in long axis.

Represented in the present collection by cultures No. 109, No. 126, and many others.

Thirty-two cultures belonging to this series have been examined in the present study. Included in this number are the authors' own isolates from a wide variety of sources together with cultures contributed by collaborators in this country and abroad. Of these, more than three-fourths regularly produce colonies conforming with the above description of the species *A. amstelodami*. Although wide variation in colony character does occur within the series, it is obvious that such variations are exceptional rather than commonplace. Accordingly, it is not believed advisable to assign or create specific or varietal names for these variations although they differ markedly from the typical *A. amstelodami* in gross appearance. An exception to this policy has been made in the case of cultures received as *A. montevidensis*, for reasons that will be considered later.

As indicated in the preceding key to the series, marked variation from the normal cultural character of *A. amstelodami* occurs along certain divergent lines.

Culture No. 5644.c (fig. 10, *C*) received from Baarn as *Eurotium repens* (Cda.) De Bary and Wor. var. *amstelodami* Vuill. (*34*) represents a variation that tends toward an almost complete suppression of the conidial phase with only an occasional small and atypical head present.

In the opposite direction, culture No. 5667.405 (fig. 10, *D*) recently received from Bliss in California (isolated from date fruits) represents a variation that produces a dense stand of conidial heads and only occasional perithecia, these being borne above rather than below the layer of crowded conidial heads.

In contrast to both of the preceding, culture No. 5681.1 (fig. 10, *E*) isolated from an old shoe, produces an extremely thin, spreading colony that bears only widely scattered perithecia or conidial heads.

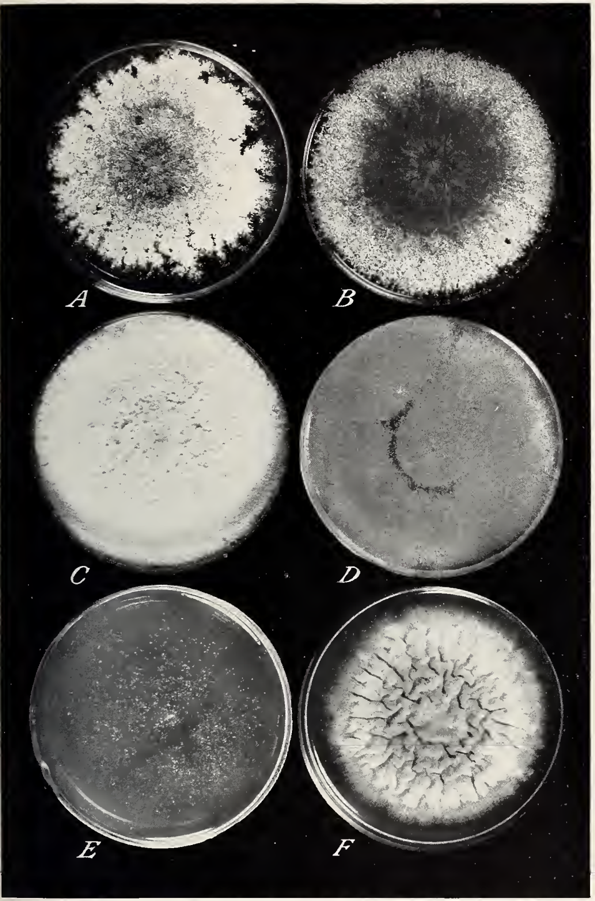

FIGURE 10.—Comparative growth of members of the *Aspergillus amstelodami* series: *A* and *B*, Typical *A. amstelodami*, showing variation in colony habit, No. 126 and No. 5669.12, respectively; *C*, No. 5644.c; *D*, No. 5667.405; *E*, No. 5681.1; and *F*, *A. montevidensis*, No. 5290. Three-week-old cultures at room temperature upon 20 percent sucrose Czapek agar. See descriptive notes pp. 22–26.

A fourth distinct variation is represented by culture No. 5290 received in 1932 from Talice as *A. montevidensis* Talice and MacKinnon (*30*). This fungus is characterized by an initially strong development of the conidial phase, and subsequently of perithecia in a felted overgrowth, which in the colony center more or less obscures the underlying conidial layer. Perithecia and conidial heads are somewhat smaller than in strains of *A. amstelodami*. Although this culture does not differ from *A. amstelodami* more widely than the variations previously noted, since it has an imputed pathogenic history and since it has been described and distributed widely under the name *Aspergillus montevidensis*, it is believed advisable to retain the name in association with this culture. Accordingly, the writers include the following emended description.

Aspergillus montevidensis Talice and MacKinnon emend. (*30, pp. 1007–1008*).

Colonies on Czapek's solution agar with 20 percent of sucrose, spreading, wrinkled and buckled (fig. 10, *F*), at first bluish green from massed conidial heads, with central area later becoming yellow from developing perithecia in a more or less tufted overgrowth of somewhat floccose mycelium; reverse in yellow-green shades to deep olive in colony center.

Colonies upon Czapek's solution agar (3 percent sucrose) restricted, radiate sulcate, with zonation evident toward the margin, central area showing coremia, perithecia few or lacking; reverse and agar very dark, almost black.

Perithecia abundant, of variable size and irregular shape with relatively few fertile asci and ascospores, late in developing, commonly 75μ to 100μ in diameter, occasionally larger; asci 10μ to 12μ in diameter; ascospores lenticular, roughened, with broad and prominent furrow flanked by low acute and irregular ridges, mostly 4.8μ to 5.2μ by 3.6μ to 4.0μ, occasional spores larger or smaller. Conidial heads very abundant, small, somewhat columnar, with few conidial chains, mostly 70μ to 80μ wide, occasionally up to 100μ; stalk up to 300μ to 350μ long, frequently very short when borne upon the aerial mycelium, broadening to a hemispherical domelike vesicular area at the apex; commonly deep green or greenish brown; vesicle mostly 15μ to 20μ in diameter, occasionally larger or smaller; sterigmata in one series relatively short and thick, 6μ to 7μ by 3μ to 3.5μ; conidia roughened, subglobose, commonly 4μ to 5μ by 3μ to 4μ, occasionally 5.5μ diameter.

Type culture isolated by Talice and MacKinnon from the tympanic membrane of the human ear (*30*). It is carried in the authors' collection as No. 5290.

LARGE-SPORED SPECIES

Under *Eurotium herbariorum* Lk., Mangin includes all of the members of the group with ascospores more than 6.6μ in long axis (*18*). In a general way this represents a very common usage in older literature beginning as far back as Corda in the 1830's. Because neither measurements nor markings of the ascospores were given, no one can fix the type of *E. herbariorum*. In general, the species in the large-spored group have both conidia and ascospores definitely larger than those in series already described. They become very conspicuous to the collector who finds the anomalous situation of an overabundance of published names and a dearth of isolations. Over a period of many years the scarcity of strains isolated in this laboratory which show ascospores larger than 7.0μ leads the authors to believe that such forms are definitely rare if not abnormal. This observation is, in effect, confirmed by George Smith (*25*). From textiles in particular he has isolated many small-spored strains but none with large spores. Possibly the present collection contains as many large-spored strains as it does because the authors have regarded them as curiosities, and for that reason retained them, whereas scores of strains of such common species as *A. repens* or *A. amstelodami* have been isolated and forthwith discarded.

In contrast to the small-spored forms, where complete duplication between large numbers of isolates is the rule, among the large-spored forms there is a marked tendency for each strain to present a somewhat different cultural picture, which is commonly coupled with differences in morphology. This would suggest that these forms are unstable and variable, but such a conclusion is refuted by their behavior in culture. To illustrate, culture No. 4481, a strain of *Aspergillus echinulatus*, has for 18 years of continuous culture in this laboratory retained its distinguishing characters, whereas the single known culture of *A. medius* has been under observation in this and European laboratories for more than 40 years without appreciable change.

Thus the problem of assigning a relatively small number of quite distinct cultures is presented. To describe each of them would merely add to the confusion already existing, hence they have been grouped somewhat, choosing either historic cultures that have become widely distributed or cultures of marked individuality as representing specific names. The homogeneity of these strains as a group is not claimed. The names *A. glaucus* and *E. herbariorum* are not identified with particular organisms in this discussion.

Aspergillus minor (Mangin) n. comb.

Synonymy: *Eurotium herbariorum* ser. *minor* Mangin, Ann. des Sci. Nat., Bot. (ser. 9) 10: 365. 1909.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, attaining a diameter of only 1 to 2 cm. in 3 weeks, irregular and wrinkled, cream colored to bluish brown, conidial heads present or lacking, small perithecia present or lacking, mostly abortive; reverse uncolored to orange maroon.

Colonies upon Czapek's solution agar with 20 percent of sucrose plane or somewhat wrinkled in the central area, spreading evenly, attaining a diameter of 8 to 10 cm. in 3 weeks (fig. 11, *A*), predominantly brick red in color becoming maroon in age, perithecia abundant and borne in a close felt of red-encrusted hyphae at the agar surface, conidial heads few in number, projecting above the perithecial layer, generally distributed over the entire colony, but occasionally concentrated in localized areas; reverse in shades of deep red brown.

Perithecia abundant, largely embedded in and obscured by a close mycelial felt at the agar surface, yellow to orange, globose to subglobose, mostly 100μ to 120μ in diameter, occasionally up to 150μ; asci 14μ to 16μ; ascospores lenticular, commonly 6.6μ to 7.4μ by 5.2μ

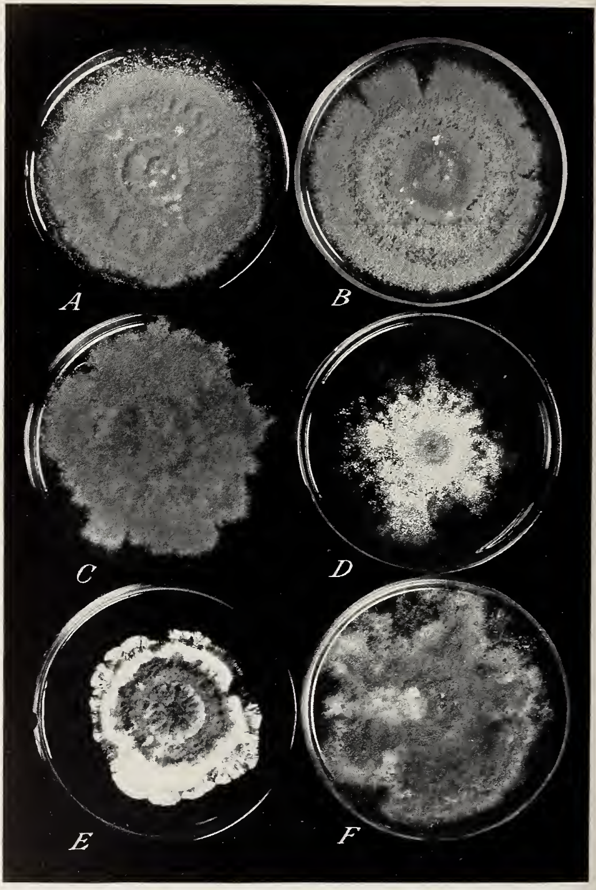

FIGURE 11.—Comparative growth of large-spored members of the *Aspergillus glaucus* group upon 20 percent sucrose Czapek agar at room temperature: *A*, *A*, *minor*, No. 5629D, 4 weeks; *B*, *A. umbrosus*, No. 4803.1914, 4 weeks; *C*, *A. echinulatus*, No. 4481, 6 weeks; *D*, *A. niveo-glaucus*, No. 7053.2, 4 weeks; *E*, *A. medius*, No. 5633.8, 6 weeks; *F*, *A. carnoyi*, No. 5612.A32, 4 months.

to 5.8μ occasionally up to 7.8μ in long axis, finely roughened in the equatorial area, ridges low and rounded or pyramidal in section, furrow generally definite, shallow but often steep-sided, V-shaped.

Conidial heads few, generally scattered, projecting above the perithecial layer, pale blue green in color, radiate, mostly 150μ to 200μ in diameter, but frequently larger; stalks smooth, pale to dark brown, mostly 700μ to 800μ in length, occasionally reaching 1 mm., broadening to 15μ to 18μ below the vescicular apex; vesicles subglobose, 30μ to 40μ; sterigmata in a single series, 8μ to 10μ by 4μ to 5μ; conidia dull green, elliptical to subglobose, mostly 6.0μ to 7.5μ and frequently 8.0μ in long axis.

Represented by culture No. 5629D, isolated from an unpainted board, as type; and by several additional cultures isolated in this laboratory. Culture No. 5619.26, received in 1937 from Oscar W. Richards, of the Spencer Lens Co., differs from the type by producing ascospores of somewhat smaller size and with less evident furrow and ridges. Thus, it may represent a strain transitional between *Aspergillus minor* and *A. ruber*. However, it is not sufficiently different from *A. minor*, either culturally or morphologically, to warrant its description as a separate species or as a distinct variety.

Mangin (*18*), in his study of the group, found specimens in his collection with ascospores less than 7.5μ in long axis, yet larger than those of the small-spored forms which he described. Having no faith in any of the descriptions existing at the time, he called the aggregate *Eurotium herbariorum* series *minor*. The cultures cited above have sufficient common characters to warrant the belief that they are variants of a common stock which may be constituted a species aggregate and appropriately attributed to Mangin, hence the usage *Aspergillus minor* (Mangin) n. comb. is proposed.

Aspergillus umbrosus Bain. and Sart. (*5, p. 267, pl. XII*).

Probable synonyms: *A. mutabilis* Bain. and Sart., Soc. Mycol. de France, Bul. Trimest. 27: 458, pl. XVII. 1911.
A. mollis Bain. and Sart., Soc. Mycol. de France. Bul. Trimest. 27: 453, pl. XVI. 1911.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, attaining a diameter of 0.5 to 1.0 cm. in 3 weeks, raised, tufted, white to orange red, bearing neither perithecia nor conidial heads; reverse colorless to orange brown.

Colonies upon Czapek's solution agar with 20 percent of sucrose, plane or somewhat wrinkled, spreading evenly or irregularly (fig. 11, *B*), reaching a diameter of 8 to 10 cm. in 3 weeks, predominantly vinaceous red to orange brown in color, consisting largely of a surface felt of sterile hyphae encrusted with orange-red granules enmeshing abundant perithecia, occasionally characterized by a loose floccose overgrowth bearing scattered perithecia, conidial heads pale blue green, widely scattered and projecting above the perithecial layer; reverse in red-brown shades.

Perithecia abundant, yellow to orange, globose to subglobose, largely embedded in a felt of sterile red-encrusted hyphae at the agar surface, occasionally borne in a loose aerial felt, mostly 120μ to

140μ in diameter, rarely up to 175μ; asci 14μ to 16μ; ascospores lenticular, mostly 7.2μ to 8.0μ by 5.6μ to 6.4μ, occasional spores up to 8.4μ in long axis, finely roughened to smooth in the equatorial areas, ridges low and generally rounded, furrow shallow, commonly V-shaped (fig. 12, *A*); conidial heads few, scattered, projecting above the perithecial layer, pale bluish green, radiate, compact, mostly 175μ to 250μ in diameter; stalks smooth, colorless to brownish, 700μ to 850μ in length, broadening to 15μ to 20μ below the expanded, domelike vesicular apex (fig. 13, *B*); vesicle 25μ to 40μ in diameter; sterigmata in a single series, 10μ to 12μ by 4.5μ to 6μ; conidia pale green, elliptical to subglobose, spinulose, mostly 7μ to 8μ in long axis, frequently larger.

Represented in the present collection by culture No. 4803.1914, received in 1925 from Dr. Florence A. McCormick, by European strains, and by several cultures isolated in this laboratory.

Culture No. B–146 contributed by Dr. Paul Simonart as an unnamed culture from the Biourge collection differs from the species as above described by consistently producing ascospores with walls entirely smooth, whereas in other characters the ascospores duplicate essentially those of No. 4803.1914. Further, No. B–146 produces colonies of lighter color than other strains under observation and may in fact represent a fungus comparable to that described as *Aspergillus mutabilis* by Bainier and Sartory.

Aspergillus umbrosus, A. mutabilis, and *A. mollis* were described by Bainier and Sartory (*4, 5*) primarily upon the basis of colony color (pigment production) and conidial apparatus, with the ascospores of *A. umbrosus* recorded as slightly less in long axis (8.0μ by 5.6μ) than those of the other species (8.4μ by 5.6μ). After careful consideration of the three descriptions and detailed study of the strains in the authors' possession showing in general the ascospore described by Bainier and Sartory, it is believed that they had at hand three cultural variants of the same species. *A. umbrosus* is retained as the species designation, as it is believed that their description of this species more adequately pictures the cultural and morphological characters of the fungi under consideration than either of the, earlier descriptions, which are left as probable synonyms.

Aspergillus echinulatus (Delacr.) Thom and Church (*31, p. 107*).

Synonymy: *Eurotium echinulatum* Delacr., Soc. Mycol. de France, Bul. Trimest. 9: 266, pl. XIV, fig. III. 1893.
E. verruculosum Vuill., Soc. Mycol. de France, Bul. Trimest. 34: 83. 1918.

Colonies upon Czapek's solution agar with 20 percent of sucrose slow-growing, plane or somewhat wrinkled, spreading irregularly, attaining a diameter of 7 to 8 cm. in 4 weeks (fig. 11, *C*), commonly mottled in appearance due to the uneven distribution of green conidial heads above the underlying orange-red perithecial layer; conidial heads bottle-green, abundant, commonly crowded in localized areas but scattered thinly throughout the remainder of the colony; perithecia abundant and borne in a felt of hyphae encrusted with red granules at the agar surface, conspicuous where not obscured by massed green heads; reverse cinnamon to deep red brown.

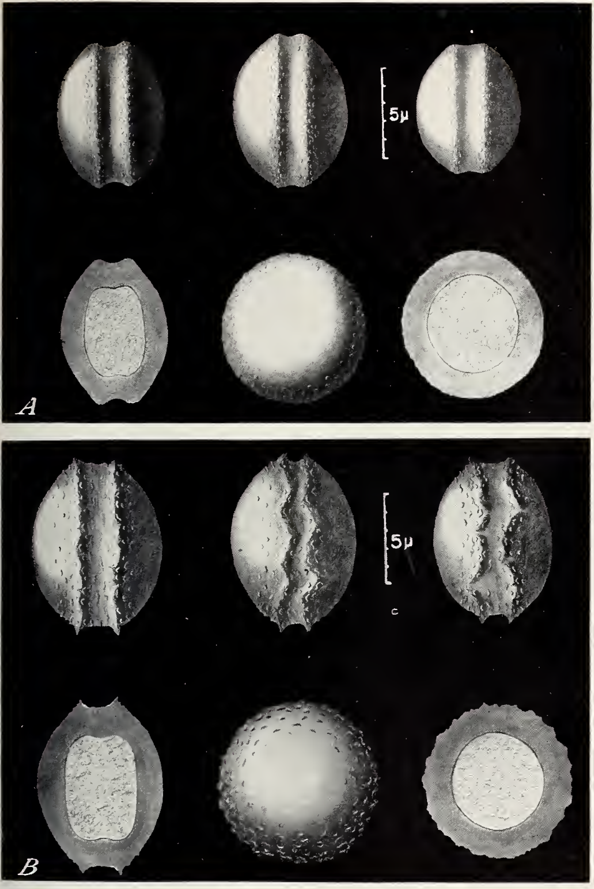

FIGURE 12.—Ascospores representative of *A*, *Aspergillus umbrosus*, and *B*, *A. echinulatus*. In each species upper left, right, and center spores represent surface profile views; lower left, optical section in profile; lower center, surface in face view; and lower right, optical section in face view.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, 1.5 to 3.0 cm. in diameter after 4 weeks, marginal area blue green from conidial heads and central portion reddish brown from an overgrowth of sterile encrusted hyphae, perithecia lacking; reverse deep orange.

Perithecia abundant, embedded in a loose felt of sterile red hyphae at the agar surface (fig. 3, *E*), yellow, globose to subglobose, mostly 125μ to 150μ in diameter, and occasionally up to 175μ; asci 18μ to 22μ; ascospores lenticular, mostly 9μ to 10μ by 6.5μ to 7.5μ, occasionally up to 11μ in long axis, conspicuously roughened in the equatorial area, furrow pronounced, broad, ridges prominent and irregular (fig. 12, *B*).

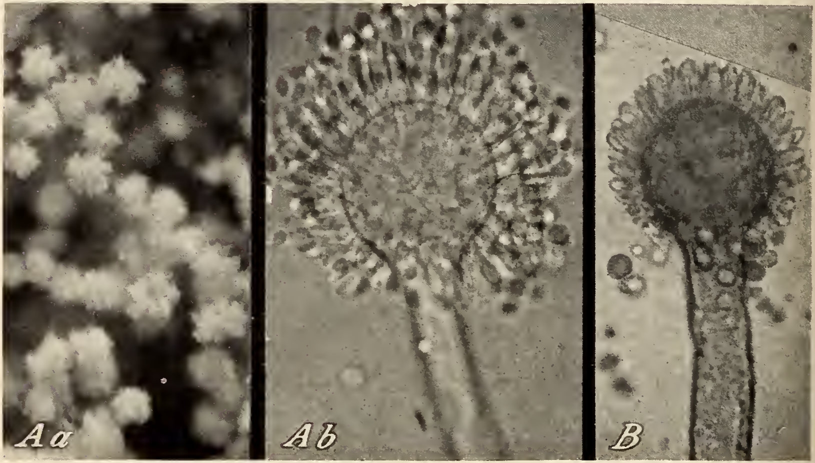

FIGURE 13.—*A*, *Aspergillus niveo-glaucus*, No. 7053.2: *a*, Conidial heads photographed by reflected light, × 25; *b*, conidial head by transmitted light, × 400. *B*, Head of *A. umbrosus*, No. 4803.1914, × 400.

Conidial heads densely crowded in localized areas and scattered throughout the remainder of the colony, bottle green in color, radiate, consisting of relatively few, long, divergent chains of conidia, commonly 250μ to 300μ in diameter but often larger or smaller; stalks smooth-walled, colorless to brown shades, commonly 700μ to 850μ in length, occasionally in excess of 1 mm., broadening from 5μ to 7μ at the base to 15μ to 20μ below the vesicular apex; vesicle 25μ to 35μ in diameter, consisting of a domelike terminus of the broadening stalk; sterigmata in a single series, not crowded, bottle-shaped, 12μ to 15μ by 5μ to 7μ; conidia elliptical, pyriform, or subglobose, echinulate, mostly 8μ to 10μ in long axis, commonly larger or smaller, extremely variable.

Represented by No. 4481 of the present collection isolated in 1921 from figs received from California. A subculture of this strain, forwarded by Miss Margaret Church about 1926, is maintained in the Centraalbureau; the two lines remain identical. No ascosporic stage has in the authors' experience been found in culture No. 5612.A28 received in 1937 from George Smith as *A. echinulatus* Delac., and obtained by him from Biourge, but its conidial development duplicates No. 4481 and it is apparently correctly assigned. Da Fonseca's and the Centraalbureau's isolations of *A. echinulatus* (see table 1) maintained at Baarn produce somewhat smaller ascospores (8μ to 9μ by 6.2μ to 7.0μ) and conidia than No. 4481, but otherwise agree essentially with the species description as given above. Somewhat further reduction in ascospore size is seen in cultures No. 5526.H11 isolated from honey, and No. 5479.A36 received from George Smith and Raistrick as *Aspergillus mongolicus* Biourge (nomen nudum). Although these are less red in color than No. 4481 and appear distinct in culture, the authors do not feel warranted in separating them as a species or variety, believing that they represent only variations from the general type designated as *A. echinulatus*.

Bainier and Sartory described *A. disjunctus* (*3*) and *A. repandus* (*4*) as vigorous species possessing ascospores 11μ by 6μ and 11.2μ by 5.6μ, respectively. Authentic cultures of these species are not now available, but the descriptions as published would seem to place them close to *A. echinulatus*.

Aspergillus medius Meiss. (*19, pp. 337–344, 353–357*).

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, 0.5 to 1.5 cm. diameter in 6 weeks, tufted, consisting of a dense growth of yellow-brown hyphae bearing neither conidial heads nor perithecia; reverse in shades of yellow brown.

Colonies upon Czapek's solution agar with 20 percent of sucrose at room temperature very slow-growing (optimum 20° C. ±), strongly wrinkled, tufted, irregular in outline, attaining a diameter of 5 to 6 cm. in 6 weeks (fig. 11, *E*), colony center deep orange red becoming yellow to white at the margin, which is characterized by bundles (becoming branching columns at lower temperatures) of hyphae bearing dark-green conidial heads in the manner of loose divergent coremia; perithecia ripening very slowly, maturing ascospores in 2 to 3 months, mostly abortive; conidial heads relatively few (more abundant and larger at lower temperatures) and borne either in coremiform masses or scattered throughout the colony; reverse in shades of orange maroon.

Perithecia scattered, mostly abortive, borne in a dense felt of orange-red hyphae, very slowly ripening, globose to very irregular in form, extremely variable in size, rarely attaining a diameter of 125μ, containing very few mature ascospores; asci 18μ to 20μ; ascospores sparingly produced, lenticular, mostly 8.8μ to 9.6μ by 6.0μ to 6.8μ, occasionally 10μ in long axis, somewhat roughened in the equatorial region, furrow broad and shallow, ridges prominent, relatively thin and irregular.

Conidial heads deep-green, radiate, compact, and of two types: Small heads 100μ to 150μ in diameter borne on loose coremiform columns, and larger heads 200μ to 250μ in diameter often scattered throughout the colony, produced more abundantly at 12° to 15° C. than at room temperature; stalks colorless to brown, mostly 250μ to 350μ in length, enlarging to 15μ to 20μ below the vesicle; vesicle subglobose, mostly 30μ to 40μ in diameter; sterigmata in a single series, crowded, short, 7μ to 8μ by 4μ to 5μ; conidia green, globose to subglobose, finely echinulate, thick walled, mostly 8μ to 10μ in diameter, but frequently larger or smaller.

Represented in this collection by culture No. 4724.45 which was received in 1924 from Raistrick, who in turn received it from the Centraalbureau. It is believed to be Meissner's original strain (*19*). Subcultures of this strain are currently maintained at Baarn and by George Smith in London. The three lines remain identical as shown by parallel cultures during the present study.

This fungus is distinguished particularly by (1) its very slow growth upon 20-percent sucrose Czapek agar at room temperature, (2) its tardiness in producing perithecia and especially in ripening ascospores, (3) its sparse production of ascospores, and (4) its formation of aerial hyphal bundles bearing conidial heads in loose coremiform fashion. Further, it grows much more rapidly upon Czapek agar containing 40 percent of sucrose than upon that containing 20 percent, a difference in concentration which does not materially affect the growth rate of such vigorous species as *A. repens* and *A. chevalieri*. Growth is much more rapid at 20° C. than at 25° to 28° C. The fungus attains a more favorable form at the lower, temperature, at which there is a heavier growth of mycelium, a more extensive development of aerial hyphal columns, and a greater production of conidial heads and perithecia.

Culturally this fungus is easily separated from all other species of the group, except possibly *A. carnoyi*.

Aspergillus carnoyi sp. nov. Biourge (ined.).

Colonies upon Czapek's solution agar with 20 percent of sucrose at room temperature extremely slow growing (optimum 18° to 20° C.), reaching a diameter of 7 to 8 cm. in 6 to 8 weeks, irregular in outline, somewhat floccose, forming a deep felt, bearing abundant perithecia and scattered conidial heads (fig. 11, *F*), orange brown in central area to orange at margin from abundant perithecia in a loose network of sterile hyphae encrusted with orange-red granules; reverse in orange-red shades.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, reaching a diameter of only 3 to 4 mm. in 6 to 8 weeks, thin, white, bearing neither conidial heads nor perithecia; reverse colorless.

Perithecia late in developing, abundant, yellow to orange, globose to subglobose, mostly 125μ to 175μ in diameter but frequently larger or smaller, borne in a loose floccose felt of sterile brown hyphae; asci 16μ to 18μ, typically 8-spored, frequently with some or all spores aborted; ascospores lenticular, variable in size and pattern, mostly 7.2μ to 8.4μ by 6μ to 6.5μ but often larger (up to 9.0μ in long axis) or smaller (down to 6.5μ in long axis), generally smooth-walled but occasionally roughened in equatorial area, generally rounded but often flattened and occasionally indented, with ridges wholly absent or indefinite, and with furrow absent or present as a trace only.

Conidial heads sparsely produced, commonly scattered, dull gray-green, radiate, compact, mostly 150μ to 200μ but often up to 250μ in diameter; stalks smooth-walled, colorless, long, commonly up to 2 mm. in length, uniform in diameter, 12μ to 18μ, to just below the vesicle, vesicle subglobose, 40μ to 50μ in diameter and occasionally larger; sterigmata in a single series, crowded, bottle-shaped, 10μ to 12μ by 5μ to 6μ; conidia globose to subglobose, echinulate, dull green, mostly 8μ to 10μ.

Species description based upon culture No. 5612.A32 received in 1937 as *Aspergillus carnoyi* Biourge from George Smith and by him earlier from Biourge. Presumably the culture is type, although Biourge's description of the species remains unpublished. The culture is distinct, not only differing in its colony character and in the length of its stalks but especially in the variable character of its ascospores. The majority of spores, although much larger, resemble those of *A. repens*, whereas spores with rough walls are occasionally produced. This culture is, therefore, somewhat of an exception to the general rule of constancy in ascospore pattern, and the variability of its spores affords one of the best characters for its identification.

Aspergillus niveo-glaucus, n. sp.

Synonymy: *A. glaucus* mut. *alba* Bloch., Deut. Bot. Gesell. Ber. 50: 248–256. 1932.
A. glaucus var. *albida*. Speg., An. del Mus. Nac. de Buenos Aires 6: 332. 1899.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, 1 cm. diameter after 4 weeks, white to cream, bearing abundant small conidial heads but no perithecia; reverse colorless to yellow brown.

Colonies upon Czapek's solution agar with 20 percent of sucrose slow growing, plane, spreading irregularly, 6 to 8 cm. in diameter after 4 weeks (fig. 11, *D*), thinning toward the margin, with mycelium in shades of yellow orange becoming cinnamon brown in age, more or less obscured by abundant white heads, and with perithecia abundant, yellow, embedded and irregularly clustered in a close felt at the agar surface; reverse yellow at margin to deep brown at colony center.

Perithecia abundant, yellow, globose to subglobose, mostly 100μ to 125μ in diameter, occasionally larger, commonly clustered, borne in an interrupted surface felt of buff to brown hyphae; asci 15μ–17μ in diameter; ascospores lenticular, mostly 7.2μ to 7.8μ by 5.0μ to 5.6μ, smooth-walled except in equatorial area, furrows broad and shallow, ridges prominent, roughened, rounded or acute and often appearing ragged. Conidial heads abundant, white (fig. 13, *A*, *a*), often becoming browned in age, radiate, mostly 250μ to 300μ in diameter; stalks smooth-walled, colorless to brown, mostly 1,000μ to 1,500μ, rarely longer, broadening to 16μ to 20μ below the vesicle; vesicle subglobose, 40μ to 50μ in diameter (fig. 13, *A*, *b*); sterigmata in a single series, crowded, 8μ to 10μ by 3μ to 4μ; conidia elliptical to pyriform, colorless, spinulose, 6μ to 8μ in long axis.

Coloniis in agaro Czapeki (sucroso 3 percent.) multo restrictis, post 4 hebdomades 1 cm. in diam., ex albis cremeis, capitula abundantia parva conidica gerentibus; peritheciis nullis; reverso ex hyalino flavidobrunneo.

Coloniis in agaro Czapeki (sucroso 20 percent.) lente crescentibus, planis, irregulariter patentibus, post 4 hebdomades 6–8 cm. in diam., marginem versus attenuatis, mycelio aurantio, vetusto cinnamomeobrunneo, plus vel minus e capitulis abundantibus albis obscurato; peritheciis abundantibus, flavis, immersis et in coacta superficiali densa irregulariter aggregatis; reverso ad marginem flavo, in centro brunneo.

Peritheciis abundantibus, flavis, globosis vel subglobosis, plerumque 100μ–125μ in diam., interdum majoribus, fere caespitosis, in coacta superficiali hypharum alutacearum vel brunnearum latis; ascis 15μ–17μ in diam.; ascosporis lenticularibus, plerumque 7.2μ–7.8μ× 5.0μ–5.6μ, regione aequatoriali excepta glabro-tunicatis, sulcis latis tenuibusque, liris prominentibus, rugosis, rotundatis vel acutis, saepe laceris, capitulis conidicis abundantibus, albis, vetustis brunnescentibus, radiatis, plerumque 250μ–300μ in diam.; stipitibus glabris, ex hyalinis brunneis, 1,000μ–1,500μ longis, rare longioribus, infra vesciculam usque 16μ–20μ dilatis; vescicula subglobosa, 40μ–50μ in diam.; sterigmatibus uniseriatis, confertis, 8μ–10μ × 3μ–4μ; conidiis ellipticis vel pyriformibus, hyalinis, spinulosis, 6μ–8μ longis.[9]

The cultural description is based upon No. 7053.2 of this collection as type; this is duplicated by No. 5633.7 received from Baarn in 1938 as *Aspergillus glaucus* Link mut. *alba* Bloch. Culture No. 5479.A35, received in 1935 from George Smith and Raistrick and by them from Biourge as *A. albidus* Speg., differs from type by consistently producing a greater quantity of conidial heads which are commonly of smaller size; the ascosporic stage of the two is indistinguishable. Apparently the name "albidus" is a manuscript use in which Spegazzini's variety (*26*) has been raised to species rank, presumably by Biourge. Blochwitz' mutation *albus* assigned to *A. glaucus* is untenable, because no definite organism can be designated as *A. glaucus*. Furthermore, the specific name *albus* applied to an Aspergillus has already been used in another section of the genus.

It is possible that Blochwitz (*10*) is right in regarding this as a mutation, but there is nothing to indicate which particular large-spored form is the parent species. The strain maintains its identity in culture and hence must be regarded as a species. Yuill (*37*), in contrast, has described white mutants of *A. nidulans* and *A. fumigatus* and has properly designated them as mutants, for they appeared in cultures under observation and are known to have been derived from typical, chromogenic strains.

ASSIGNMENT OF NAMED CULTURES STUDIED

Table 1 is presented primarily as a summary of the authors' assignment of named cultures of the *Aspergillus glaucus* group maintained in well known culture collections. In addition it reveals something of the history and relationship of these cultures. The table is based upon detailed comparative cultural and morphological study of each of the strains listed and the similar study of an even greater number isolated in this laboratory over a period of many years or contributed as unnamed specimens by individual collaborators. Only cultures bearing names as received are included.

VARIATION

The genetic history of the Aspergilli is an untouched field. Separation into large groups is easily made, definitely enough to include all but a few strains. Within these groups, variation is so great that differentiation of species requires critical examination and comparison of material, including extensive culture. Unwilling to undertake this, Neill (*22*) disposed of the whole group with yellow perithecia by calling them all *A. glaucus*. On the other hand, Mangin (*18*), with the same problem of variability before him, found the ascospores sufficiently distinctive and dependable to warrant proposing to separate *A. amstelodami* and *A. chevalieri* as separate species in the *A. glaucus* group, leaving certain aggregates admittedly inadequately studied. Bainier and Sartory (*3, 4, 5*), working with members of these ill-defined species, used color production as the basis for separation.

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[9] Latin diagnosis prepared by Edith Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry.

TABLE 1.—*Allocation of named cultures of the Aspergillus glaucus group contributed for this study by culture collections and individual collaborators*

Subgroup or species	Culture No.	Name as received	No. as received	Source		Identification, Thom and Raper	Remarks
				Immediate	Secondary		
Aspergillus repens series	4640. 404	*A. mollis* Bain. and Sart.	404	Da Fonseca	Balnier collection	*A. repens* (Cda.) de Bary	Probable source of No. 5612.Ac58 and No. 5632.3784.[1]
	4640. 407	*A. scheelei* Bain. and Sart.	407	do	do	do	
	5612. 13	*A. repens* (Cda.) Sacc.	13	L. S. T. M.[2]	Smith's isolation	do	Typical *A. repens*.
	5612. Ac36	*A. glaucus* Lk.	Ac36	do	C. B. S	do	
	5612. Ac58	*A. mollis* Bain. and Sart.	Ac58	do	do	do	Same as No. 5632.3784.
	5612. Ac60	*A. scheelei* Bain. and Sart.	Ac60	do	do	do.[3]	Same as No. 5633.14 and No. 5632.3790.
	5612. Ac61	*A. disjunctus* Bain. and Sart.	Ac61	do	do	do	Same as No. 5632.3776.
	5632. 3776	do	3776	N. C. T. C.[4]	do	do	Same as No. 5612. Ac61.
	5632. 3784	*A. mollis* Bain. and Sart.	3784	do	do	do	Same as No. 5612. Ac58.
	5632. 3790	*A. scheelei* Bain. and Sart.	3790	do	do	do	Same as No. 5633.14 and No. 5612.Ac60.
	5633. 13	*A. ruber* (Spieck. and Brem.) Thom and Church.		C. B. S.[5]	Da Fonseca	do	
	5633. 14	*A. scheelei* Bain. and Sart.		do	N. C. T. C	do	Same as No. 5612.Ac60 and No. 5632.3790.
	5633. 17	*A. repens* (Cda.) Sacc.		do	C. B. S. isolation	do	
	5633. 18	do		do	Thompson	do	
	5633. 19	do		do	Sartory	do	
	5644. 3	*Eurotium herbariorum* Lk. ("green flame" variant).		do	Barnes	do	Typical *A. repens*.
	5644. 7	*E. repens* (Cda.) De Bary and Wor. forma *alpha* Mangin.		do	Mangin	do	Do.
	5479. A33	*A. dierckxii* Biourge[6]	A33	L. S. T. M	Biourge (No. 631)	do	Not typical (see pp. 8, 12).
	5343	*A. pseudoglaucus* Bloch.		C. B. S	Blochwitz	*A. pseudoglaucus* Bloch	Type culture.
	5479. A38	*A. fumigatoides* Bain. and Sart.	A38	L. S. T. M	Biourge (No. 642)	do	Duplicates No. 5343 in culture and morphology.
	5479. A27	*A. profusus* (Bain.) Hann.	A27	do	Biourge (No. 621)	do	Floccose, few heads, and perithecia.
	5633. 16	*A. profusus* Hann		C. B. S	Zach	do	Duplicates No. 5479.A27.
	4724. 46	*A. novus* Wehmer	46	Raistrick	C. B. S. (ex Wehmer)		Apparently intermediate between *A. repens* and *A. ruber* (see p. 13).

See footnotes at end of table.

TABLE 1.—*Allocation of named cultures of the Aspergillus glaucus group contributed for this study by culture collections and individual collaborators*—Continued

Subgroup or species	Culture No.	Name as received	No. as received	Source		Identification, Thom and Raper	Remarks
				Immediate	Secondary		
Aspergillus ruber series	4724.39 [7]	*A. ferrugineus* Fuckel	39	Raistrick	C. B. S. (ex v. Oyen)	*A. ruber* (Spieck. and Brem.) Thom and Church.	Duplicates No. 5612.Ac39 and No. 5632.3777.
	5612. 6	*A. ruber* Spieck. and Brem.	6	L. S. T. M	Smith's isolation	do	Typical *A. ruber*.
	5612.A17	*A. repandus* Bain. and Sart.	A17	do	C. B. S. (ex Church)	do	
	5612. A18	*A. umbrosus* Bain. and Sart.	A18	do	C. B. S	do	
	5612. Ac39	*A. ferrugineus* Lk.	Ac39	do	do	do	Duplicates No. 4724.39 and No. 5632.3777.
	5632. 3777	*A. ferrugineus* Fuckel	3777	N. C. T. C	do	do	Duplicates No. 4724.39 and No. 5612.Ac39.
	5633. 11	*A. ruber* (Spieck. and Brem.) Thom and Church.		C. B. S	Pollacci, 1930	do	
	5633. 12	do		do	Pollacci, 1931	do	
	5644. 2	*Eurotium herbariorum* Lk. ("flame" variant).		do	Barnes	do	
	5612. 23	*A. ruber* (Spieck. and Brem.)	23	L. S. T. M	Smith's isolation	do	Culture duplicates No. 5600A (p. 18).
	5612. A25	*A. glaucus* group	A25	do	J. L. Yuill	do	Culture duplicates No. 134 (p. 18).
	5612. 96	*A. herbariorum* ser. *minor* Mangin.	96	do	Smith's isolation	do	Culture similar to No. 5629E (p. 18).
	5612. A29	*A. repandus* Bain. and Sart.	A29	do	Biourge (No. 624)	do	Do.
	5479. A31	*A. lovainensis* Biourge	A31	do	Biourge (No. 628)	do	Strain distinct in culture.
Aspergillus chevalieri series.	5612. 109	*A. chevalieri* Mangin	109	L. S. T. M	Smith's isolation	*A. chevalieri* (Mangin) Thom and Church.	Typical *A. chevalieri*.
	5633. 23	*A. amstelodami* Mangin		C. B. S	da Fonseca	do	Do.
	5633. 26	*A. chevalieri* var. *multiascosporus* Nakazawa et al.		do	Nakazawa	do	Not typical *A. chevalieri*.[8]
	5479. A30	*A. oriolus* Biourge	A30	L. S. T. M	Biourge (No. 626)	do	Ascospores finely roughened.
	5633. 25	*A. chevalieri* Mangin		C. B. S	C. B. S. isolation	do	Do.
	5612. 107	*A. chevalieri* Mangin [9]	107	L. S. T. M	Smith's isolation	*A. chevalieri* var. *intermedius* n. var.	Transitional between *A. chevalieri* and *A. amstelodami*.
	5612. Ac5	*A. chevalieri* Mangin	Ac5	do	J. H. V. Charles	do	
	5632. 3789	*A. repens* (Cda.) Sacc.	3789	N. C. T. C		do	
	5644. 4	*Eurotium herbariorum* Lk. ("blue conidial" variant).		C. B. S	Barnes	do	
	109	*A. glaucus* Lk.		Westerdijk (1909)		*A. amstelodami* (Mangin) Thom and Church.	Typical *A. amstelodami*.
	4876. 17	*E. repens* (Cda.) De Bary and Wor. var. *amstelodami* Vuill.	b	Westerdijk (1926)	Vuillemin	do	
	5612. 50	*A. chevalieri* Mangin	50	L. S. T. M	Smith's isolation	do	

Aspergillus amstelodami series.	5612.75	*A. amstelodami* Mangin	75	do	do	do	
	5633.21	do		C. B. S	Church (No. 126)	do	
	5633.1945	do	1945	N. C. T. C	do	do	Do.
	5644b	*E. repens* (Cda.) De Bary and Wor. var. *amstelodami* Vuill.	b	C. B. S	Vuillemin	do	Culture abnormal but ascospores typical.
	5644c	do	c	do	do	do	Conidial heads suppressed (p. 24).
	5644d	do	d	do	do	do	Typical *A. amstelodami*.
	5644.1	*E. herbariorum* Lk. ("normal" strain).		do	Barnes	do	Do.
	5664.6a	*E. herbariorum* Lk. ("c yellow" variant).		do	do	do	Atypical. Perithecia irregular in form.
	5290	*A. montevidensis* Talice and MacKinnon.		MacKinnon	Talice and MacKinnon's isolation.	*A. montevidensis* Talice and MacKinnon.	Type culture.
	5633.24	do		C. B. S	Talice	do	Do.
Aspergillus minor	5633.10	*A. ruber* (Spieck. and Brem.) Thom and Church.		do	Church	*A. minor* (Mangin) n. comb.	Ascospores larger than in *A. ruber*.
Aspergillus umbrosus	5612.A37	*A. umbrosus* Bain. and Sart.	A37	L. S. T. M	Biourge (No. 639)	*A. umbrosus* Bain. and Sart.	Considered typical.
	5633.9	do		C. B. S	Church	do	Do.
	B-146	Unnamed	B-146	Simonart	Biourge	do	Ascospores smooth, colonies less red than No. 5633.9.
Aspergillus echinulatus	5612.A15	*A. echinulatus* Delacr.	A15	L. S. T. M	C. B. S. (ex Church?)	*A. echinulatus* Delacr	Typical.
	5612.A28	do	A28	do	Biourge (No. 622)	do	Conidial stage typical, no perithecia.
	5633.4	do		C. B. S	Church (No. 4481)	do	Typical, duplicate of No. 5612.A15.
	5633.5	do		do	Da Fonseca	do	Ascospores smaller (p. 33).
	5633.6	do		do	C. B. S. isolation	do	Do.
	5479.A36	*A. mongolicus* Biourge	A36	L. S. T. M	Biourge (No. 637)	do	Atypical strain (p. 33).
Aspergillus medius	4724.45	*A. medius* Meiss.	45	Raistrick	C. B. S. (ex Bierberg)	*A. medius* Meiss	Typical.
	5612.Ac45	do	Ac45	L. S. T. M	C. B. S. (ex Church?)	do	Do.
	5633.8	do		C. B. S	Church (No. 4724.45)	do	Do.
Aspergillus carnoyi	5612.A32	*A. carnoyi* Biourge	A32	L. S. T. M	Biourge (No. 630)	*A. carnoyi* n. sp. Biourge (in ed.).	Type culture.
Aspergillus niveo-glaucus	5479.A35	*A. albidus* Speg.	A35	do	Biourge (No. 635)	*A. niveo-glaucus* n. sp	Conidial heads more abundant and smaller than in type No. 7053.2.
	5612.A16	*A. glaucus* mut. *albus* Bloch.	A16	do	C. B. S. (ex. Blochwitz)	do	Same as No. 5633.7.
	5633.7	do		C. B. S	Blochwitz	do	Duplicates type strain.
Aspergillus argillaceus	5479.A34	*A. argillaceus* Biourge	A34	L. S. T. M	Biourge (No. 632)	Possibly *A. repens*	Nonascosporic (p. 14).

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[1] A culture received from Da Fonseca was carried as *A. mollis* Bain. and Sart. by the Centraalbureau until 1933 (*11*).

[2] L. S. T. M. = London School of Tropical Medicine; cultures in custody of George Smith.

[3] This probably represents Bainier and Sartory's conception of *Aspergillus scheelei* Bain. and Sart. Cultures are predominantly yellow, and ascospores commonly show a trace of furrow (see p. 12).

[4] N. C. T. C. = The National Collection of Type Cultures, Lister Institute of Preventative Medicine, London.

[5] C. B. S. = Centraalbureau voor Schimmelcultures, Baarn.

[6] *Aspergillus dierckxii* was sent by Biourge to Raistrick and Smith as a new species and was included by Gould and Raistrick in their study of the crystalline pigments of the *Aspergillus glaucus* group (*16*).

[7] Culturally and microscopically this strain is duplicated by No. 5612.Ac39 and No. 5632.3777. All have come indirectly from Baarn and probably represent lines from the same initial isolation despite the citation of one as *A. ferrugineus* Lk.

[8] Ascospores smooth-walled, bearing low erect crests rather than thin flexuous ones. Crests occasionally lacking and spores resemble those of *A. repens*.

[9] Received with following notation: "Close to *Aspergillus chevalieri*—differs in having smooth, small conidia and ascospores somewhat larger than type."

They cited ascospore measurements as incidental details in description. Because they appear to have had only a few strains in culture and to have described them all as new species, the task presented few difficulties to them; but unfortunately without the original cultures, no one has ever been able to identify their species with confidence. Raistrick and his colleagues (*1*, *13*, *16*, *23*), using cultures named as received and including an unpublished series from Biourge, have given quantitative figures as to pigment production and pigment mixtures for each culture listed by the name on the tube, without reporting comparative study of the morphology found.

Careful checking of their tabulations against the present record, based upon ascospores in strains as received from them, indicates that the relative quantities of these pigments in the particular strain are not indices of species affiliation. It would thus appear fair to conclude that there is little correlation between morphological detail and biochemical activity as quantitatively determined, although the general nature of such activities usually appears to be essentially similar in the related group. A particular biochemical activity at a special level is then commonly a strain character and not an attribute of a species aggregate. If the worker has found an organism valuable for his particular purpose, it becomes most important that he maintain it in pure culture and at an optimum activity, which as a rule must be determined for each strain separately.

It is clear that wide mycelial or colony variations may be found in nature between strains that retain the ascospore characters of the species. Several such groups have been held in this laboratory for 30 years or more and furnish convincing evidence that Mangin was justified in using the ascospore as the stable and readily determinable integrating character. Some of these forms retain colony characters in fairly stable form through many transfers on many laboratory substrata and over a period of years. Others grown in various substrata in Petri-dish cultures show sectors or other irregularities in colony habit, which can be picked out and established as strain variants that maintain their special characteristics in continuous culture.

The possibility that variants of similar nature might be induced by chemical stimulation led Thom and Steinberg (*33*) to select from the authors' collection certain strains that had remained fairly constant for many years. Of this group they subjected strain No. 126 of *A. amstelodami*, found apparently stable for 30 years, to extensive chemical stimulation (*28*). These experiments yielded two groups of effects: (1) A progressive reduction in the production of conidial heads and of perithecia, and (2) a great increase in the mass of vegetative mycelia. In no case was spore production completely suppressed although reduced to inconspicuous quantity. The conidia and ascospores when examined were found to have retained the size and markings characteristic for the species, whereas the mass of vegetative mycelium became excessive and formed a floccose or cottony mass entirely different in colony appearance from the original.

At this point they reversed the procedure and applied stimulants designed to reestablish spore production (*29*). As a result, the final cultures show abundant green heads with normal conidia and numerous perithecia with ascospores retaining the characters of the species. In routine laboratory examination these extreme variants would not suggest the original strain of *A. amstelodami,* although both types of variants produce conidia and ascospores typical of the species.

In 1928, Barnes (*6*) studied the possibility of heat in inducing variations. He used a strain reported as *Eurotium herbariorum* (Wigg.) Link, which had been isolated and maintained in his laboratory for several years without apparent changes. Unfortunately, no description of his normal strain was given, but a strain received from Westerdijk as "Barnes' normal strain" proves to be identical with *A. amstelodami* (Baarn strain, the authors' No. 109; or the authors' own strain No. 126 as used by Thom and Steinberg). No reasons were offered for the original identification.

Barnes described a series of experiments in which the spores of his organism were subjected to heat under varied conditions, then planted. From the resulting colonies he described 11 variants 6 of which are available in the Centraalbureau. The authors' transfers of these have been checked against the descriptions in Barnes' paper and obviously represent his isolations.

These strains were designated by him as follows, and the present identification follows his vernacular name:

No. 5644.2 "Flame" variant is *A. ruber.*
No. 5644.3 "Green flame" is *A. repens.*
No. 5644.4 "Blue conidial" is *A. chevalieri* var. *intermedius.*
No. 5644.5 "Creamy" is a pale yellowish strain of Yuills' genus *Cladosarum.*
No. 5644.6 "D Brown" is *A. ustus.*
No. 5644.6a "C Yellow" is *A. amstelodami.*

Of these, "C Yellow" shows the ascosporic pattern and differs little from Barnes' normal strain or the authors' *A. amstelodami.* "Flame," "Green flame," and "Blue conidial" show ascospores of the *glaucus* group but differ markedly in pattern. Among large numbers of induced variations, changes in the characters of the ascospore have not been found during this study." D Brown" produces no ascospores but develops the hülle cells and conidial heads characteristic of the *Aspergillus ustus* group. These four forms belong to ubiquitous species quite abundant as contaminants where plant material is handled. Such contamination is not satisfactorily excluded by the work reported.

"Creamy" presents a different problem. The possibility that this "*Cladosarum*" was actually derived from the "normal" *A. amstelodami* is not excluded. Proliferation of the sterigmata in the head of Aspergilli, especially among the *A. glaucus* lot, is very common. The branches produced sometimes are found sterile but usually become diminutive stalks with very small vesicles and groups of sterigmata producing normal spores. The Yuills' *Cladosarum olivaceum* (the authors have the type culture) was found as a conspicuous variant or contaminant in their culture of *A. niger* (*38*). The colony, stalk, vesicle, primary sterigmata, and initial secondary sterigmata are produced as in *A. niger,* then instead of chains of conidia with the newest or youngest conidia at the bases of the chains and connected directly with the sterigmata, chains of cells are produced that replicate the sterigmata; occasionally a chain is interrupted by one cell producing a group of new chains, thus acting as a primary sterigma.

These chains of cells lengthen not at the base as in *Aspergillus* but at the distal end. In spite of prolonged search, which shows that the cells toward the outer ends of such chains lose definiteness as sterigmata, the authors cannot confirm the finding of single terminal conidia on each chain as reported by the Yuills. This morphological picture is repeated by Barnes' "creamy" strain, which must therefore be interpreted in terms of Yuills' genus. Only the two isolations are known thus far. Their failure to produce true spores and their rapid loss of vitality observed in the present cultures lead to the hypothesis that they are both variants from *Aspergillus* and belong to the "monster" type of organisms that fail to survive in competitive environments. Rare occurrences of the "monster" type such as these can hardly be regarded as permanent members of the fungus flora, even though the individual can be kept viable by regular vegetative transfer. It is doubted, therefore, whether generic designation is warranted.

INFLUENCE OF TEMPERATURE

Temperature of incubation is a major factor governing the growth of members of this group. And upon their growth response to temperature they can be divided roughly into three groups as follows: (1) Species with an optimum temperature of approximately 20° C.; (2) species with an optimum temperature of approximately 30° C. or more; and (3) species with an optimum temperature of below 25° C.

The first of these groups, or what may be conveniently termed the low-temperature group, includes all of the large-spored species with the exception of *A. minor*. In *A. echinulatus*, *A. niveo-glaucus*, *A. umbrosus*, *A. medius*, and *A. carnoyi*, maximum growth occurred at 18° to 22° C. And it is interesting to note that in each of these species greater growth occurred at 12° C. than at 30° C. (fig. 14, *A*, *a–c*). They are thus definitely low-temperature organisms. It is noteworthy that the group includes all but one of the large-spored species, whereas it includes none of the more vigorous small-spored species. Further, it is probably significant that the one large-spored species not showing optimum growth at 20° C.±, namely *A. minor*, is obviously a species that is transitional between the large-spored forms and the *A. ruber* section.

Although there is no evidence that the large-spored species are abundant in nature, a fact that is discussed elsewhere in this publication (see p. 26), it is suggested that they could possibly be isolated more frequently by employing an initial incubation temperature of 15° to 20° instead of 25° to 28° C., as is commonly done..

The second of these groups, which may be termed the high temperature group, includes *A. repens*, *A. amstelodami*, and *A. chevalieri*. These are the most successful species of the entire *glaucus* group, judged by the frequency with which they are isolated (see discussion of species p. 27) and interestingly enough, they are all characterized by small ascospores rarely exceeding 5μ in the long axis. At 20° C. the growth of these species was definitely less rapid than at 30° C. Whereas at 12° C. the growth of *A. repens* was very much reduced (fig. 14, *B*, *a–c*), that of *A. amstelodami* was extremely slight, and *A. chevalieri* failed to grow. This latter observation is confirmatory of Mangin's description of *A. chevalieri* (*18*) as a high-temperature organism.

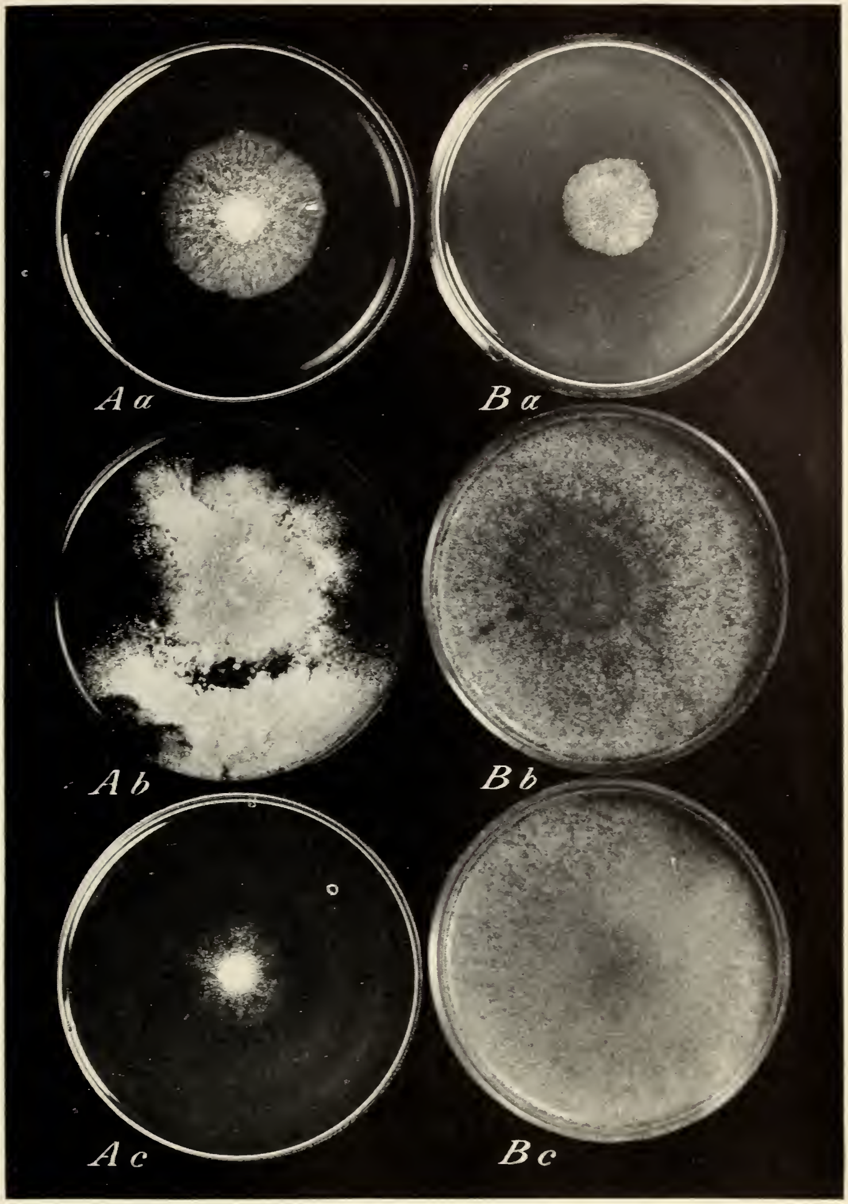

FIGURE 14.—Comparative influence of temperature upon the growth of the large-spored species *A. niveo-glaucus* and the small-spored species *A. repens: A, a–c, A. niveo-glaucus* incubated for 3 weeks at 12°, 20° to 22°, and 30° C., respectively; *B*, **a–c**, *A. repens* incubated in similar fashion. Cultures grown upon 20 percent sucrose Czapek agar.

The third group, of which the optimum temperature is intermediate between the other two, includes *A. ruber* and *A. minor*. Of these, *A. ruber* is by far the most abundant in nature and, perhaps significantly, bears ascospores on the one hand smaller than those of *A. minor* but larger than those of *A. repens*. And, as noted above, from the standpoint of ascospore size, *A. minor* is transitional between *A. ruber* and the large-spored species. Growth of these species is about the same at 20° C. and 30° C., but is much reduced at 12° C.

Thus a definite correlation appears to exist between ascospore size, optimum temperature, and the success of the species in nature. The most successful species produce small ascospores and grow best at a temperature level in excess of room temperature; the least successful bear large ascospores and grow best at temperature levels a little below room temperature; whereas the moderately successful species grow best at the level of room temperature and bear ascospores of a size intermediate between those of the other two groups.

It may be noted, as a general rule applying to the whole *A. glaucus* group, that in any given species, when grown at different temperatures, the ratio of conidial heads to perithecia increases at lower temperatures of incubation and decreases at higher levels. In this connection it is of interest to note that aside from temperature, the quantity of conidial heads can be likewise increased by adding peptone to the 20-percent sucrose Czapek agar regularly employed.

SUMMARY

The *Aspergillus glaucus* group has been interpreted as an omnipresent and nearly omnivorous lot of fungi showing green heads and yellow perithecia upon the most diverse substrata. The collection studied contains organisms isolated by the authors and series from the great culture collections of the world, many of those bearing the specific names under which they have been discussed in the taxonomic and biochemical literature.

After comparative culture under a wide range of conditions, the strains studied have been brought together into a series of aggregate species each characterized by the production of ascospores within a particular size range and bearing typical markings.

The species aggregates recognized are *A. repens*, *A. ruber*, *A. chevalieri*, *A. amstelodami*, *A. minor*, *A. umbrosus*, *A. echinulatus*, *A. medius*, *A. carnoyi*, and *A. niveo-glaucus*, n. sp. Within these aggregates, for each of which a type is described, the additional species and varieties recognized are *A. pseudoglaucus*, *A. chevalieri* var. *intermedius*, n. var., and *A. montevidensis*. The usages represented by strains received under particular names in culture are tabulated to show their place in the arrangement of species proposed. On the basis of variation studies, forms showing the ascospores of a series but differing in colony morphology and details of activity are regarded as variants, not taxonomic varieties.

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